Defence Research and Development Canada
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Development of LC/MS Methods to be used in Decontamination Research with CW Agents

Final Report

D. Noot

Prepared by:
Vogon Laboratory Services Ltd.

Contract Scientific Authority: M. Mayer, DRDC Suffield

Defence R&D Canada

Contract Report

DRDC Suffield CR 2010-147

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contract # W7702-09R230

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Abstract

Analytical methods using liquid chromatography-tandem mass spectrometry for the detection of CWAs in decontamination formulations were developed and validated. Various parameters were investigated, including mass spectrometer parameter optimization, investigation of ionization matrix effects, chromatographic separation, use of internal standard type compounds, linearity, carry over and precision. The sampling design for decon experiments was also investigated and modified to ensure accurate results. The methods are suitable for the agents GF and GD, and the decon formulations RSDL and British Decon using F54.

The final methods allow detection of agents in decon formulation samples using dilution as the only sample preparation step ("dilute and shoot"). As such, the methods will provide accurate identification and quantitation of agents in real time to test decon formulation efficacy.

Generic protocols for adapting the developed methods for use with other agents and decon formulations were also prepared.

Executive Summary

Title: Development of LC/MS methods to be used in Decontamination Research with CW Agents

Introduction

Analytical methods for the quantitation of chemical warfare agents (CWAs) in decontamination formulations were required for research purposes. Liquid chromatography – tandem mass spectrometry (LC-MS/MS) is a highly specific and sensitive technique, allowing samples to be analysed with minimal sample preparation. As such, the technique is a good fit for the timely analysis of CWA degradation in complex decon formulations.

Results

LC-MS/MS methods were developed, allowing for direct analysis of agents in decon formulation solutions with no sample preparation other than dilution. The dilution step performs two functions:

- quenching of the decon reaction to provide a snapshot of the agent concentration in time,
- reduces the decon solution to an appropriate level where matrix effects in the ionization source are eliminated.

This report details the work performed to fully develop and validate the analytical methods as well as address issues in the decon experiment sampling process. Generic protocols are also presented for future work with agents and decon formulations not studied in this contract.

Significance

These methods will allow DRDC staff to perform research on GD and GF in RSDL and the British Decon formulations to determine efficacy under different conditions. Various parameters in the decon experiment sampling design and analytical method were investigated and optimized to ensure that a decrease in agent concentration is due to actual decon and not some other process. The generic protocols provide the steps to be taken to create methods compatible with different agents and decon formulations for future research.

Future Plans

Future work to improve the ability to perform decon research includes:

- identifying break down products of CWAs in decon reactions and developing methods to quantify them ,
- improving the decon formulation sampling procedure by customizing the Gilson automated liquid handler to increase efficiency and accuracy while maintaining the benefits of reduced handling of agents for researchers,
- investigate the potential for use of other analytical instruments present at DRDC, including the 6130 single quad MS system using APCI ionization and the evaporative light scattering detector.

Definitions

6460	model number of the Agilent mass spectrometer used for this project
ACN	acetonitrile
AJS	Agilent Jet Spray
APCI	atmospheric pressure chemical ionization
CWA	chemical warfare agent
DI H2O	deionized lab grade water
DAD	diode array detector
ESI	electrospray
ESTD	external standard
F54	phase-stable microemulsion decontamination formulation
GD	Soman, a CWA
GF	Cyclohexyl sarin, a CWA
IDL	instrument detection limit
IPA	isopropyl alcohol
ISTD	internal standard
LC	liquid chromatograph
MeOH	methanol
MPEG	methoxypoly(ethylene glycol)
MRM	multiple reaction monitoring
MS	mass spectrometer
NH4Ac	ammonium acetate
QQQ	triple quadrupole (or tandem quadruple) MS
%RSD	percent relative standard deviation
RSDL	reactive skin decontaminant lotion
TEP	triethyl phosphate
TBP	tributyl phosphate
TPP	tripropyl phosphate

Outline of Actions based on Objectives

Objective 1

"The first objective of this contract is to develop robust, well characterized, scientifically sound LC/MS methods for quantifying the concentration of various CWA in complex decontamination mixtures for both the 6130 MS and the 6460 QQQ instruments. The work will be to determine the LC/MS methodology for two chemically related CW agents (GF and GD) with one decontamination matrix, RSDL (Reactive Skin Decontamination Lotion). The optimized LC/MS methods need to be used on both the 6130 MS single quad and the 6460 MS triple quad. As such, method development will be undertaken on the 6460 QQQ, and then adapted for the 6130 MS single quad, if possible."

Approach: studies were performed with RSDL using GF extensively and GD to a lesser degree.

All specific tasks listed in the contract were completed, with the exception of those listed below.

Specific Tasks	Completed	If no, Reason
1.16 Adaption of the method for the 6130 MS single quad where possible	no	Based on work performed with the triple quad (tandem) MS, and knowing that matrix effects are an ionization source phenomenon and that the single quad MS system is less specific and sensitive than the triple quad, it was determined that the single quad would not likely be a useful tool for testing decontamination solutions. With the Scientific Authority's approval, it was decided to not pursue this action.
1.17 Preparation of templates for work lists, methods and reports for Agilent MassHunter and ChemStation software	yes for MH no for CS	ChemStation templates are specific to the 6130 single quad MS, and as no work was done on the single quad, no templates were generated.

Objective 2

"The second objective is to develop a generic LC/MS protocol to rapidly screen potential decontamination formulations using LC/MS and both the 6130 MS and the 6460 QQQ. This would be developed for a single, representative CW agent (either GF or GD, based on the results from Objective 1) and tested using a decontaminant matrix different than RSDL.

As well, it is expected that the method development undertaken in Objective 1 will be used as the basis for the generation of the generic methodology. The optimized LC/MS methods need to be used on both the 6130 MS single quad and the 6460 MS triple quad. As such, any further method development will be undertaken on the 6460 QQQ, and then adapted for the 6130 MS single quad, if possible."

Approach: The British Decon solution (and F54 matrix) was chosen as a model to work with for Objective 2. GF was the agent studied extensively.

All specific tasks listed in the contract were completed, with the exception of those listed below.

Specific Tasks	**Completed**	**If no, Reason**
2.5 Adaption of the generic protocol for the 6130 MS single quad where possible	no	See explanation for 1.16.
2.6 Testing of the generic protocol using one CW agent and one decontamination matrix on the 6130 single quad	no	As the protocol was not adapted for use with the 6130, it was not tested on that instrument.
2.9 Preparation of templates for work lists, methods and reports for Agilent MassHunter and ChemStation software	yes for MH no for CS	See explanation for 1.17.
2.11 A draft report of the method validation suitable for submission to a peer reviewed journal		The work performed for objective 2 is not suitable for publication in a peer reviewed journal at this time. Therefore no draft manuscript was prepared.

General Tasks Supporting Objectives 1 & 2

All specific tasks listed in the contract were completed.

Results and Discussion

Objective 1

1.1 Literature review

A colleague in DRDC (Dr. Paul D'Agostino) was contacted regarding literature references as he maintains a collection of all relevant CWA papers. Two papers were used more extensively than others:
"Recent advances and applications of LC-MS for the analysis of chemical warfare agents and their degradation products – A review" by P.A. D'Agostino (1),

and

"Rapid Screening procedures for the hydrolysis products of chemical warfare agents using positive and negative ion liquid chromatography-mass spectrometry with atmospheric pressure chemical ionization" by Read and Black (2).

Internal DRDC documents were used to gather background information on RSDL and F54 decon formulations (not referenced). Publically released information was also used (3 & 4, respectively).

1.2 Assistance in preparation of CW standards and dilutions

Assistance was provided on several occasions for decon experiment solution and standard preparation.

1.3 Selection of an appropriate internal standard

The alkyl phosphate compounds TEP, TPP and TBP were investigated for use as internal standards.

Full scan spectra of TEP, TPP and TBP are shown in figures 1.3a – c. Note that ammonium (NH4) adducts are not formed with these compounds, which is different than the G agents GF and GD. Figure 1.3a shows two fragmentor voltages for TEP. At 60V the predominant ion is [M+H]+ at 183, while fragment ions are seen at 120V(bottom).

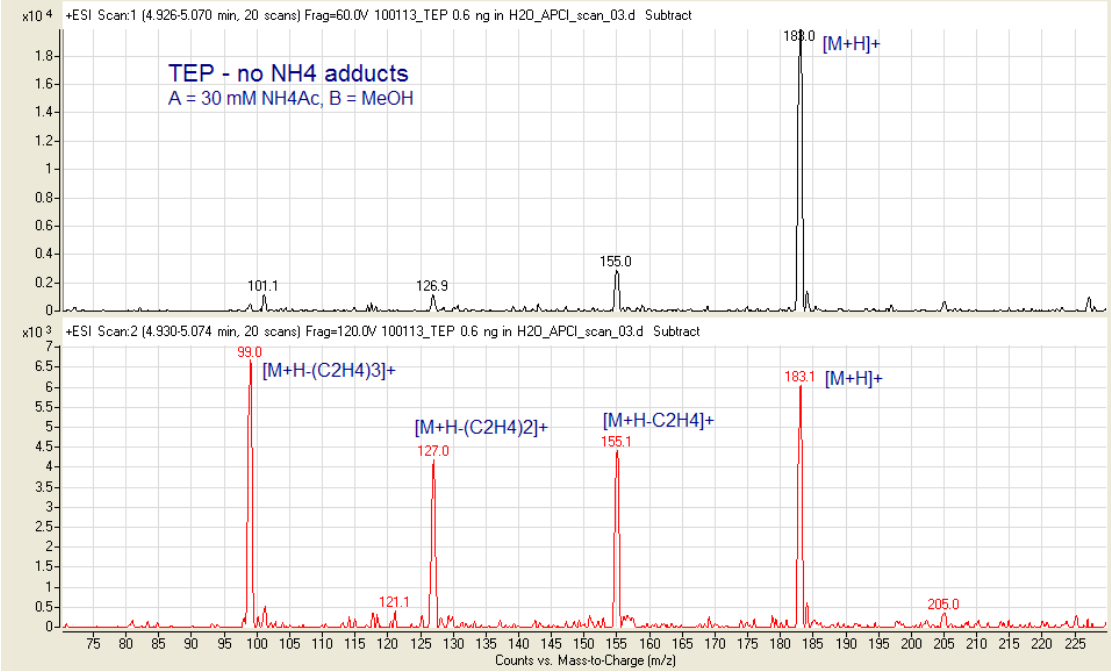

Figure 1.3a – full scan spectra of TEP at fragmentor voltages of 60V and 120V

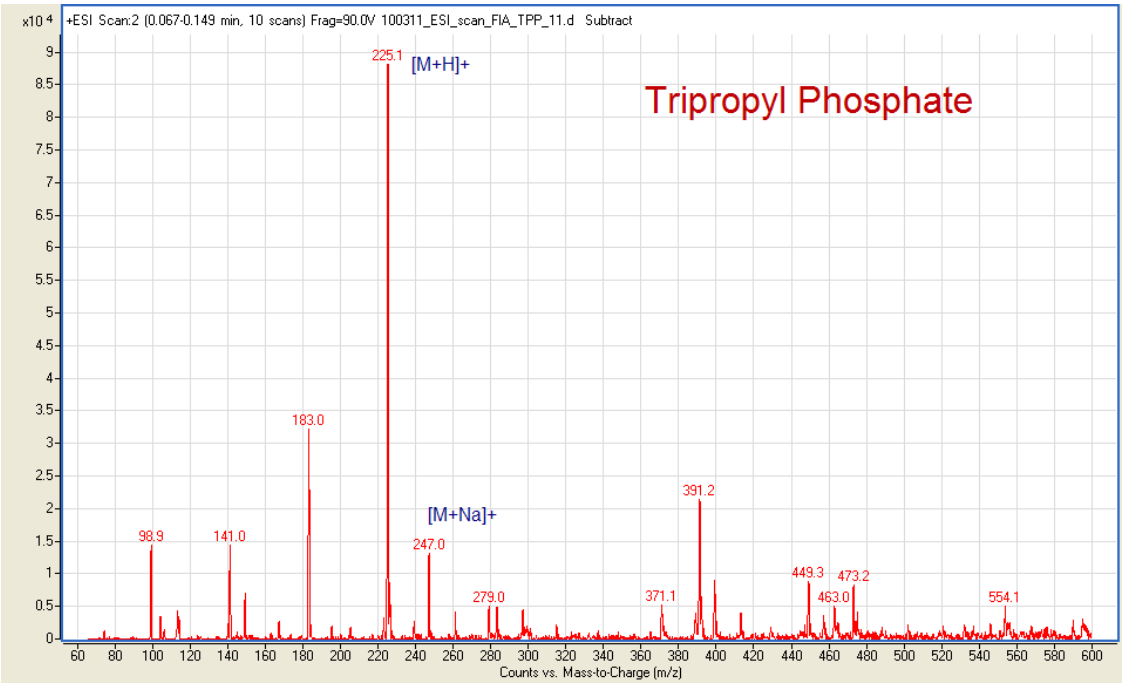

Figure 1.3b – full scan spectra of TPP

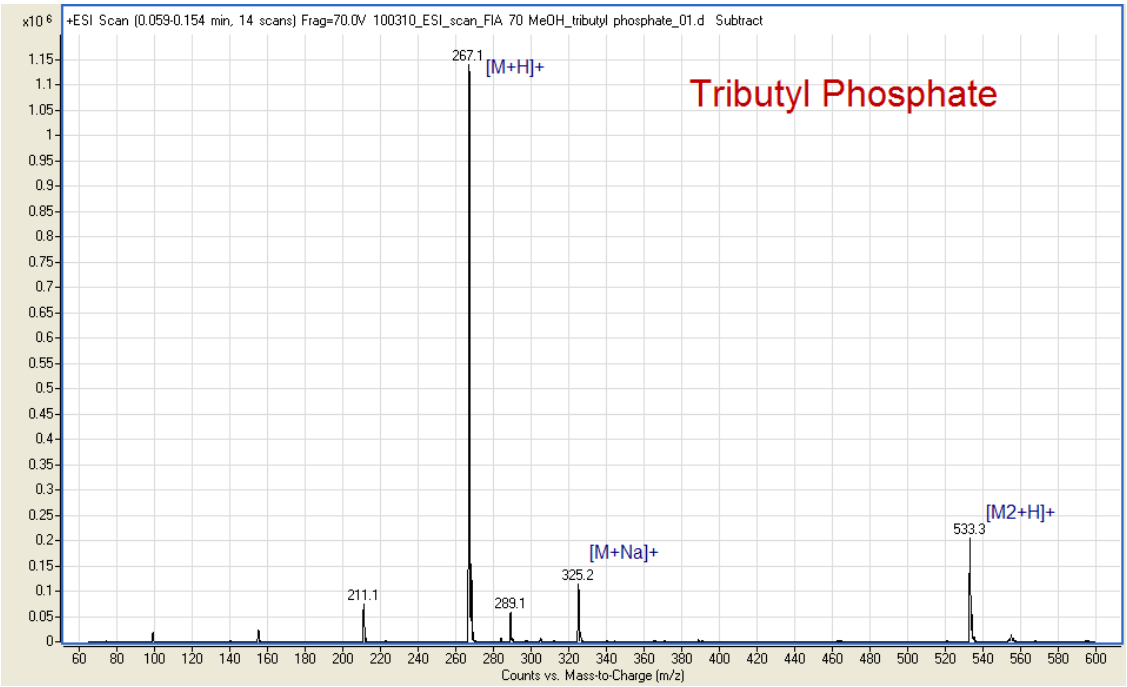

Figure 1.3c – full scan spectra of TBP

TEP typically elutes before GF with the RDSL matrix components, making it a good indicator of ion suppression using ESI for that matrix. As such, TEP can be used to ensure that decon experiment final dilutions are at an appropriate level where no ion suppression is occurring. If TEP peak areas (and therefore recoveries) are consistent, then any changes to the agent concentrations can then be attributed to decon rather than ion suppression.

TPP typically elutes after the agents GF and GD, and is therefore not a good indicator of ion suppression. It is, however, a good compound to be used to monitor the entire sample preparation and handling process. By adding TPP at the beginning of the sample preparation, it can be considered to be a "surrogate" compound. Monitoring the recovery of a surrogate provides information on all aspects of the entire method from sample preparation to instrumental analysis. An ideal surrogate behaves similarly to the target compounds while not interfering with their analysis, and TPP fits this description. Poor surrogate recovery (and good recovery of the TEP added at to the final dilution showing no ion suppression and valid instrumental analysis) will indicate losses due to sample handling, e.g. insufficient mixing, phase separations in the vial, solution losses, etc.

TBP elutes later than TPP, well after the gradient reaches 100% MeOH. It also exhibits a higher degree of carry over (data not shown). It is therefore not recommended for use in decon experiments.

Recommendations - given the difference in ion suppression for TEP and TPP compared to GF, and given the good accuracy of properly diluted decon solutions compared to compounds in solvent (analytical standards),

- It is not recommended that the ISTD calculation method be used. Rather, use the ESTD method of calculation and manually monitor recoveries of TEP and TPP as discussed below.
- Add an appropriate amount of TPP (see section 1.7) to the decon solution prior to adding the agent. Subsequent dilutions will bring the level down to the final applicable range. Monitor the recovery of this surrogate to gauge sample preparation and dilution procedures.
- Add an appropriate amount of TEP (see section 1.7) to the final solutions (i.e. the final dilutions that will be analysed on the LC-MS system) in a decon experiment. By adding TEP just prior to instrumental analysis, the recovery can be used to indicate problems with the instrumental analysis (final volumes, amount injected,) and most importantly, ion suppression due to inadequate dilution of matrix.

1.4 Optimization of the mass spectrometer

CW agents and internal standards were optimized using a manual process or using MassHunter Optimizer. It should be noted that Optimizer did not initially provide suitable results for GF due to the fact that the agent tends to form adducts. Using 5 mM NH4Ac in DI H2O as the aqueous mobile phase produced a strong ammonium adduct ([M+NH4]+) which proved to be a stable ion to use as the precursor for MS/MS analysis. In many injections during the course of the contract, both the [M+H]+ and [M+NH4]+ precursor ions produced very similar results in terms of accuracy. The [M+NH4]+ transitions are 17 times more intense (see table 1.4a), and therefore the lower intensity [M+H]+ transitions were not included in the final MRM method.

Injected	**[M+NH4]+ to [M+H]+ Area Ratio**	
	ESI	**APCI**
GF 0.0046 ng	15.7	
GF 0.023 ng	17.2	20.6
GF 0.12 ng	17.4	16.2
GF 0.6 ng	17.5	16.5
GF 3 ng	17.1	14.9
average	*17.0*	*17.0*

Table 1.4a – relative intensity of [M+NH4]+ to [M+H]+ for GF by ESI and APCI

Negative ion electrospray (ESI) did not produce appreciable signal for GF, GD and TEP. As such, positive ion mode was used.

The final optimized values for each compound are presented in table 1.4b.

Compound	Precursor (Fragmentor ,V)	Product Ion (Collision Energy, V)	Typical Relative Abundance
GF	198.1 (60 V) [M+NH4]+	99 (4 V) 181.1 (0 V)	100% 24.7%
GD	200.1 (55 V) [M+NH4]+	85 (2 V) 183.1 (0 V)	100% 23.3%
TEP	183.1 (70 V) [M+H]+	99 (15 V) 127 (6 V)	100% 31.3%
TPP	225.1 (58 V) [M+H]+	99 (12 V) 141 (4 V)	100% 26.5%
TBP	267.2 (58 V) [M+H]+	99 (12V) 155 (4 V)	100% 22.3%

Table 1.4b – optimized MS parameters for GF, GD, TEP, TPP and TBP

Graphic representations of the MRM transitions are shown in figures 1.4a - e. Dwell times were set to 90 ms for each transition.

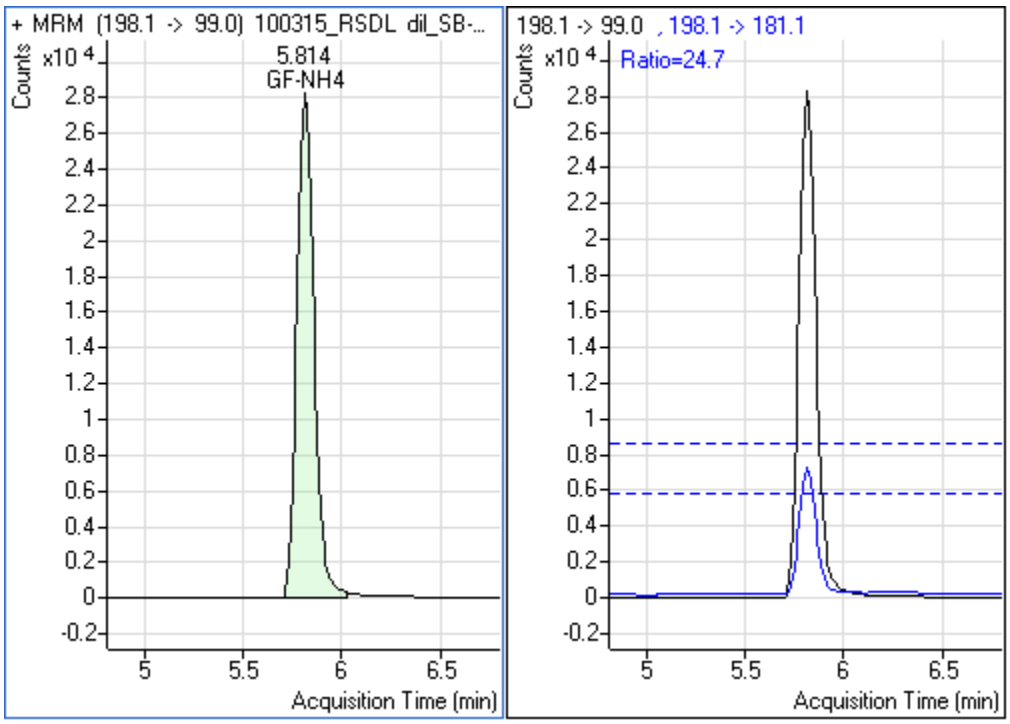

Figure 1.4a – GF MRM transitions

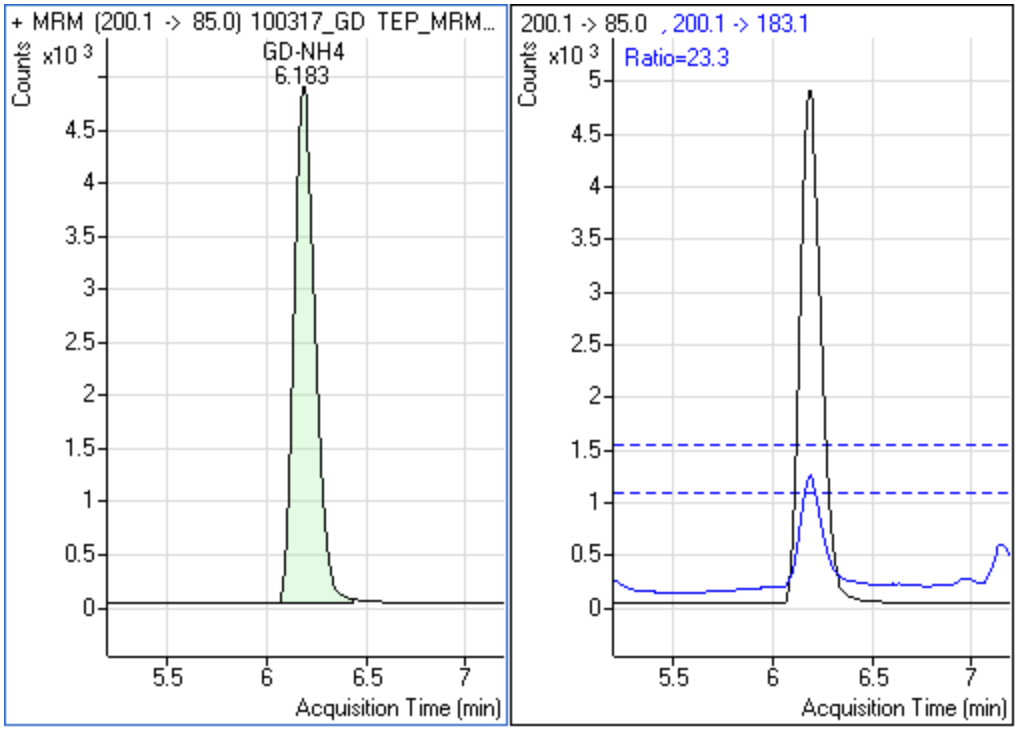

Figure 1.4b – GD MRM transitions

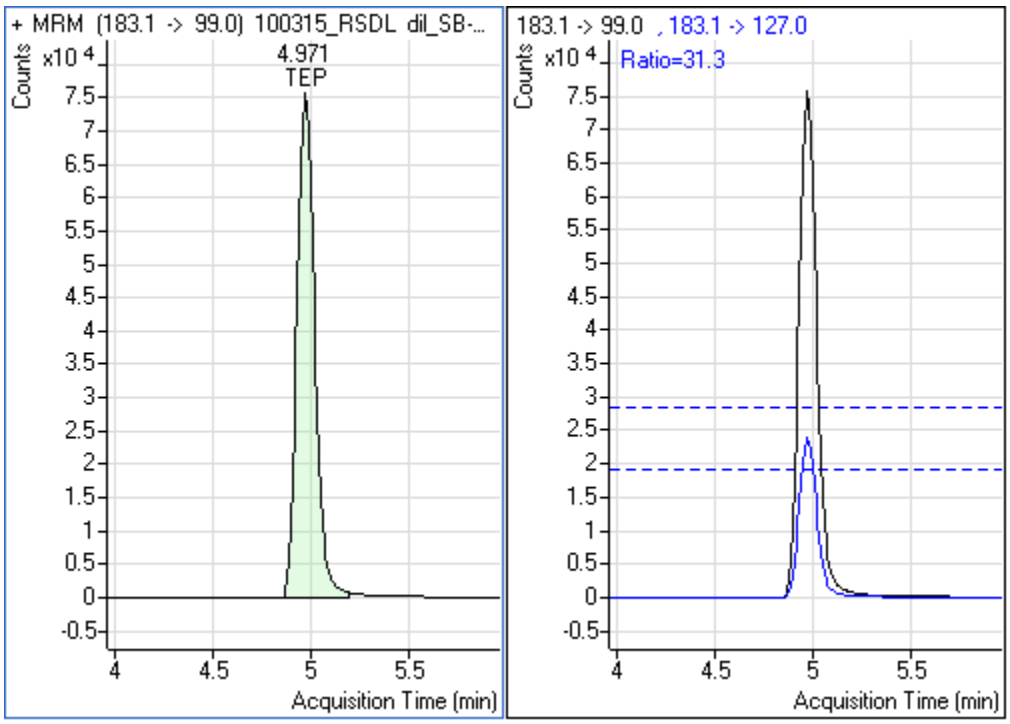

Figure 1.4c – TEP MRM transitions

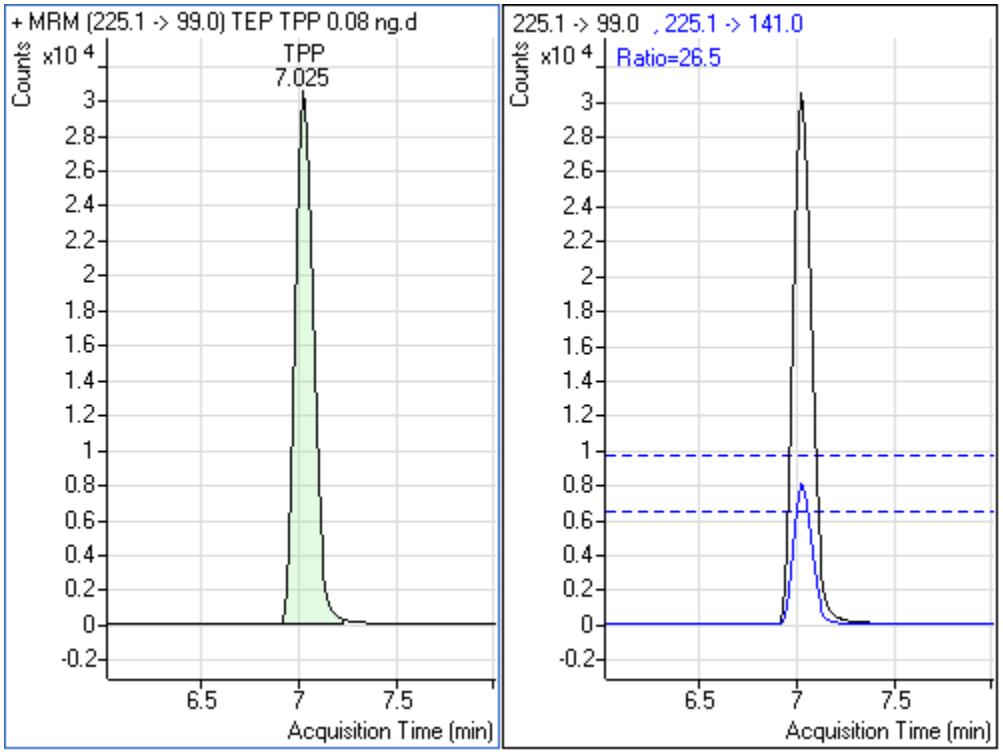

Figure 1.4d – TPP MRM transitions

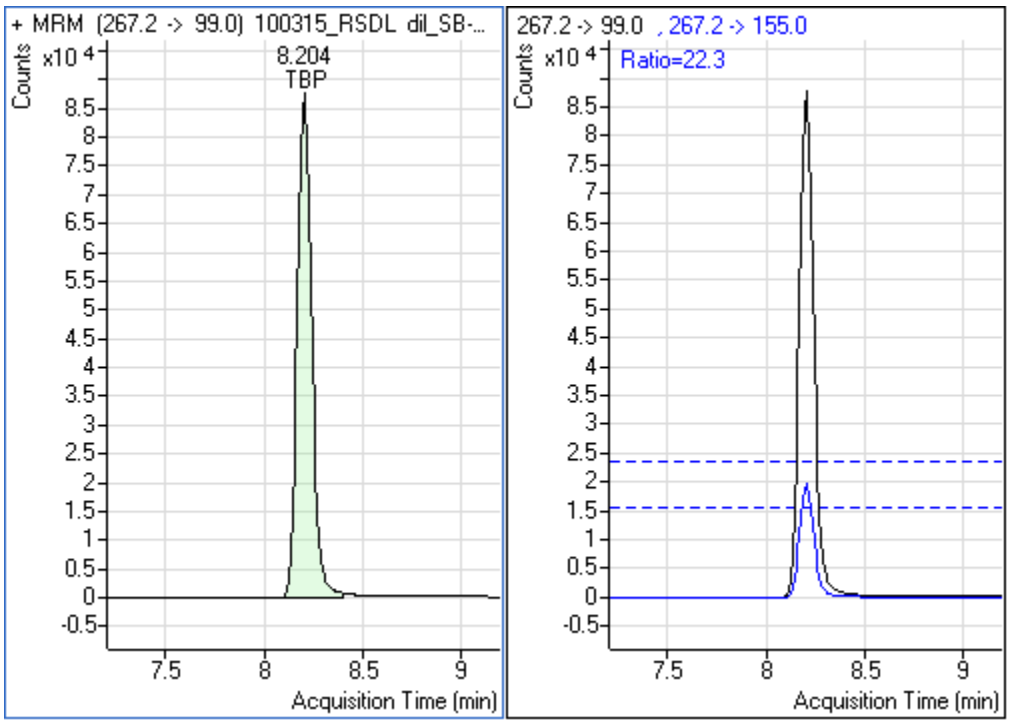

Figure 1.4e – TBP MRM transitions

Source conditions were optimized for the chromatographic conditions and target compounds. Final optimized values for ESI-AJS are as follows:

Column	Zorbax SB-C18, 2.1x50mm, 1.8µ
LC Conditions	0.3 mL/min flow rate with gradient of 20 – 100% B
	A = 5 mM NH4Ac, B = MeOH
Source parameters – ESI with Agilent Jet Spray, positive mode	
Gas Temp	300 °C
Gas Flow	4 L/min
Nebulizer	50 psi
Sheath Gas Temp	250 °C
Sheath Gas Flow	10 L/min
Capillary	2500 V
Nozzle Voltage	500 V

APCI can also be used if necessary. APCI source conditions were optimized in a preliminary fashion, and as such, further optimization may be necessary.
Optimized source conditions for APCI are as follows:

Column	Zorbax XDB-C18, 4.6x50mm, 1.8µ
LC Conditions	0.65 mL/min flow rate with gradient of 20 – 100% B
	A = 5 mM NH4Ac, B = MeOH

Source parameters – APCI, positive mode	
Gas Temp	300 °C
Gas Flow	4 L/min
Nebulizer	40 psi
Vaporizer Temp	350 °C
Corona	10 µA
Capillary	3000 V

1.5 Development of an MRM method for the 6460 QQQ

MRM methods were generated for GF and GD using the optimized values for each CWA and ISTD compound, for both ESI and APCI. See Annex 1 for the MRM method listings. Methods are located on the 6460 workstation in the "D:\MassHunter\Methods\!Decon Experiments" folder.

1.6 Develop chromatographic separation of the components of interest

Use of the DAD was potentially beneficial for investigating the elution profile of the matrix and active components in RSDL. Various wavelengths in the UV range were used, however no signal was observed, even for high concentrations of RSDL injected. As such, use of the DAD was discontinued and the mass spectrometer was used in full scan mode to detect matrix components.

Using the MS in full scan mode, the MPEG components of RSDL that make up the "solvent" were detectable. A mass range of 105 – 1200 amu was used. A lowest mass of 105 was chosen as there were several ions in the blank at 101 amu and below, and so these were excluded from runs investigating where RSDL components elute. A high mass range of 1200 was used as the highest mass of the RSDL solvent matrix was found to be approximately 1100 amu.

Three different LC columns were investigated with RSDL:

- Zorbax SB-Phenyl, 2.1x100 mm, 3.5 µm
- Zorbax XDB-C18, 4.6x50 mm, 1.8 µm
- Zorbax SB-C18, 2.1x50 mm, 1.8 µm

The retention of GF and TEP was compared to the retention of the MPEG solvent used in RSDL in an effort to minimize ion suppression. Figures 1.6a shows the overall elution pattern for MPEG, the first and last eluting MPEG components, and the %B gradient used for the three columns tested (20 to 100 %B from 1 to 8 minutes). It can be seen that the Phenyl column shows the highest degree of separation of the MPEG matrix components.

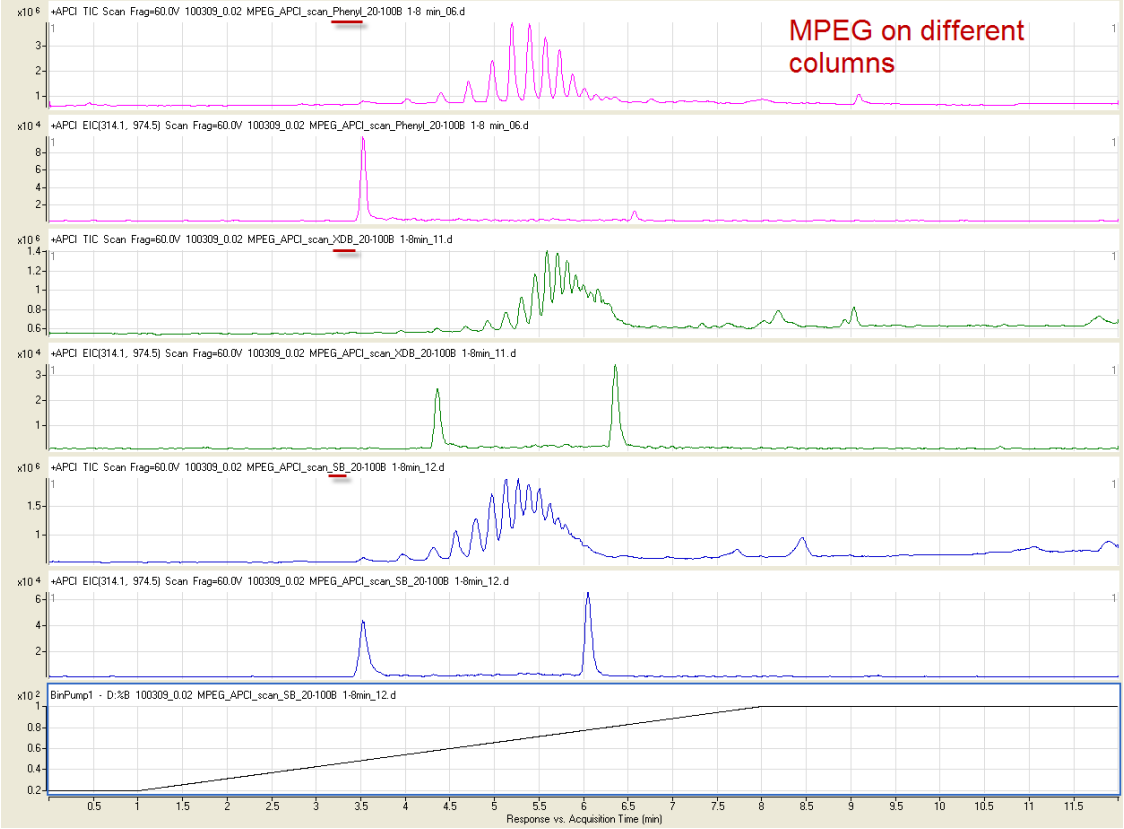

Figure 1.6a – MPEG elution pattern for Zorbax Phenyl, Eclipse XDB C18 and SB-C18 columns

Figures 1.6b – d show elution profiles for individual columns, as well as MRM traces for TEP and GF on that column. It can be seen that for the Phenyl column, TEP elutes before the majority of the MPEG while GF elutes in the same region as the major MPEG components. For the XDB column, TEP elutes in the MPEG region while GF elutes after MPEG. Both TEP and GF elute in the MPEG region on the SB column, although the majority of MPEG has eluted by the time GF elutes.

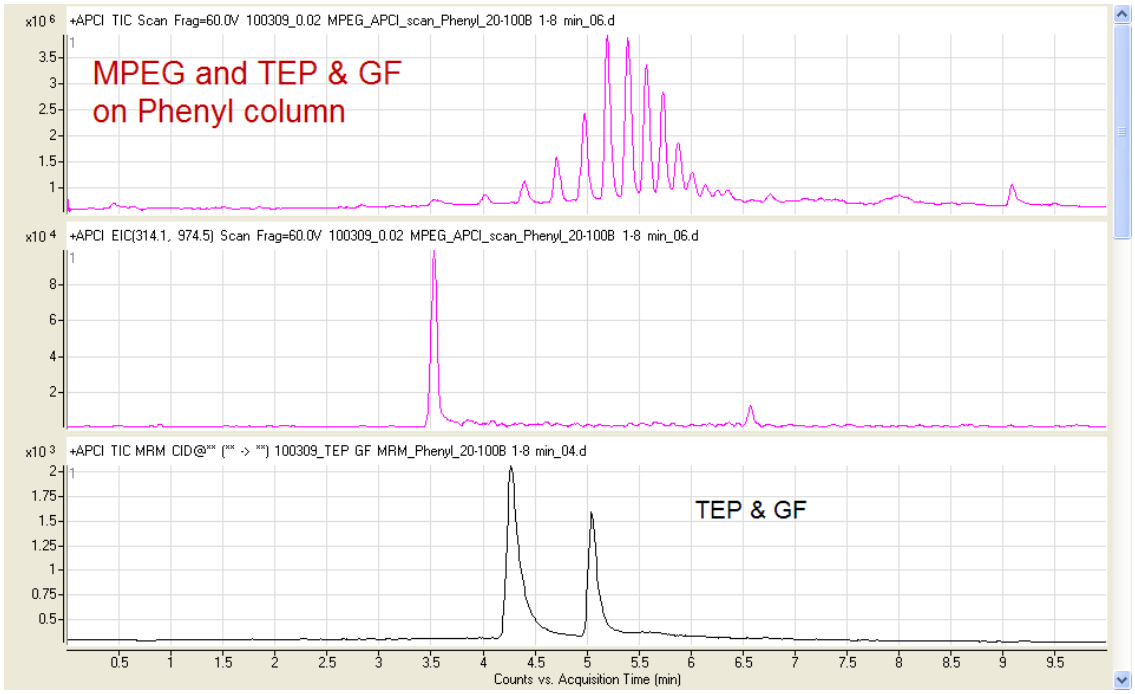

Figure 1.6b – MPEG, TEP and GF elution on Phenyl column

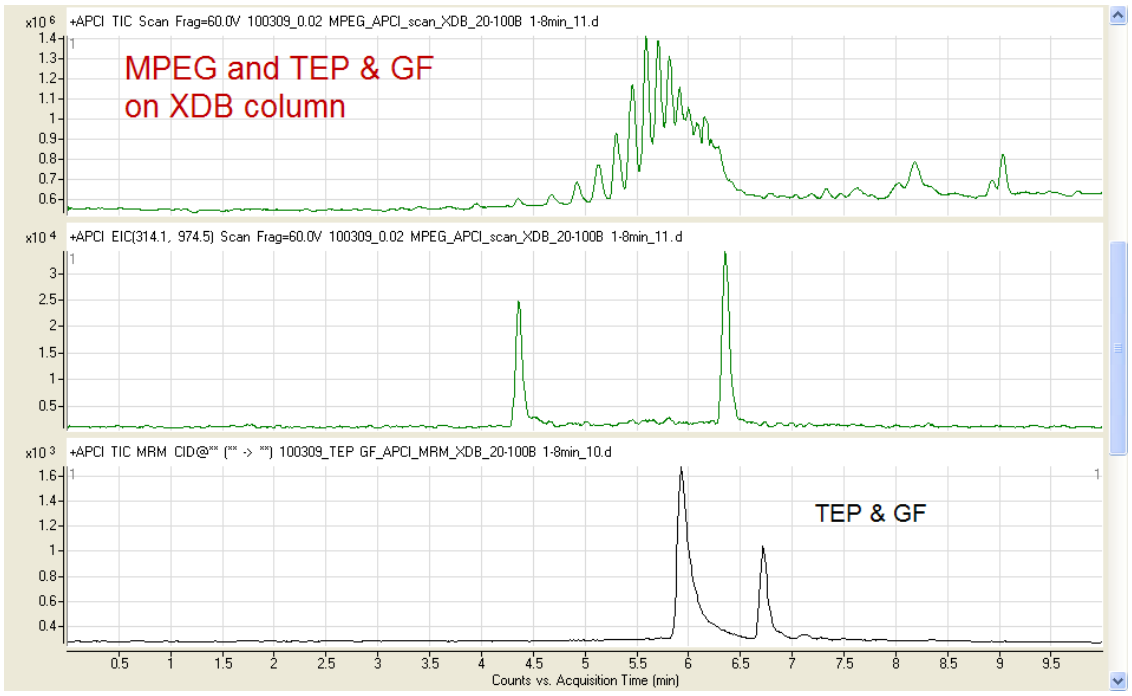

Figure 1.6c – MPEG, TEP and GF elution on XDB-C18 column

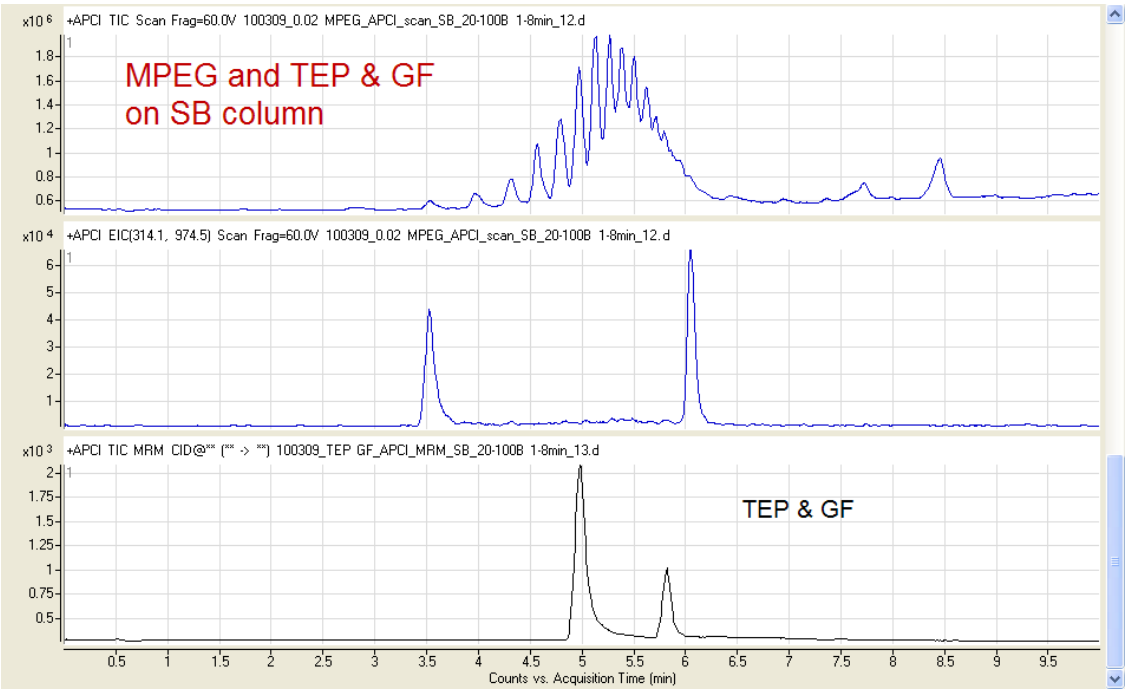

Figure 1.6d – MPEG, TEP and GF elution on SB-C18 column

The final choice of column was determined by the amount of ion suppression found with both RSDL and F54 decon solutions (see Results sections 1.8 & 2.2) as well as compatibility with different ionization modes. The SB column was chosen for ESI methods and the XDB column for use with APCI.

The effect of injection volume was investigated. The G agents are susceptible to hydrolysis and therefore should be dissolved in ACN rather than water. Injecting samples in such a "strong" solvent compared to the initial mobile phase conditions (20%B) can result in poor peak shape and shifting retention times. The final MRM methods use an injection volume of 1 µL to avoid such chromatographic problems.

As was mentioned earlier, strong ammonium adducts were seen (and are used) for the GF and GD. The concentration of NH4Ac in the mobile phase was investigated. Figures 1.6e & f show that the ESI response for TEP and GF decreases as the concentration of NH4Ac increases (5, 30 & 50 mM). Figures 1.6g & h show that there is no significant difference on TEP and GF response in APCI between 10, 20 and 30 mM NH4Ac.

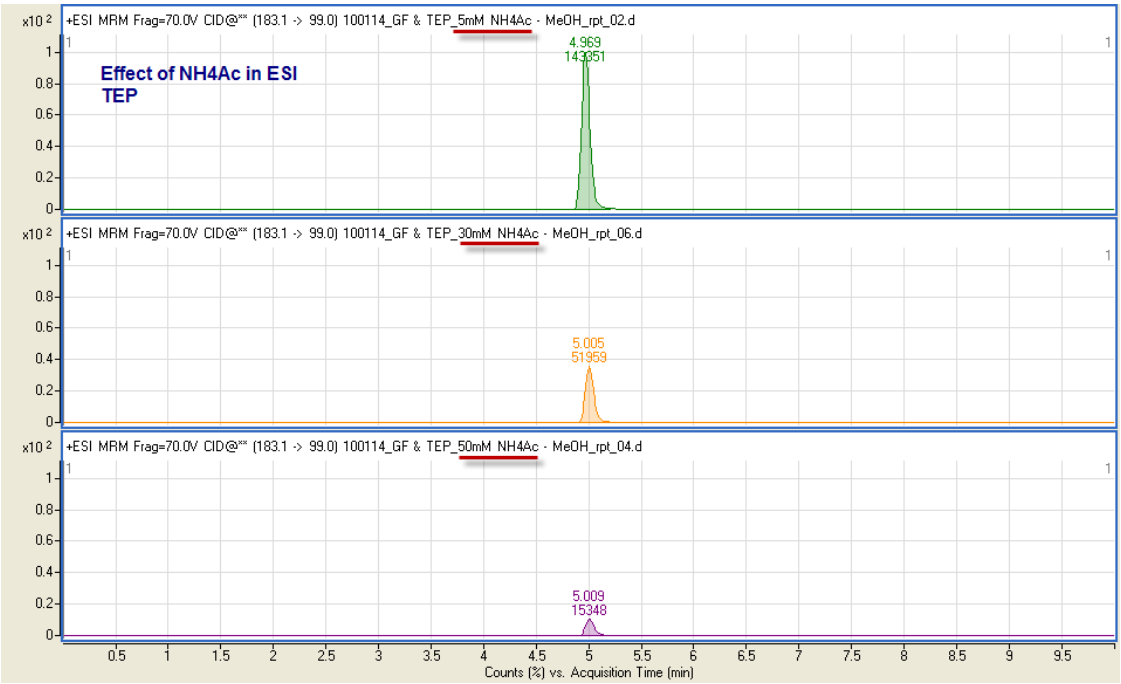

Figure 1.6e – the effect of NH4Ac concentration on TEP response in ESI

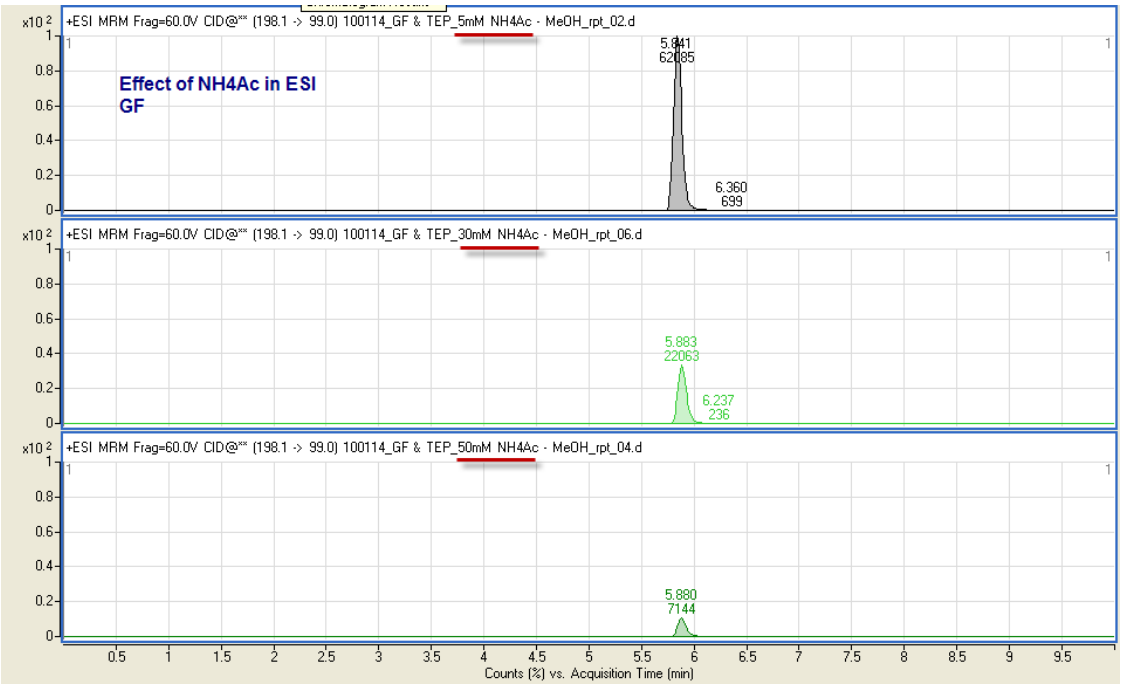

Figure 1.6f – the effect of NH4Ac concentration on GF response in ESI

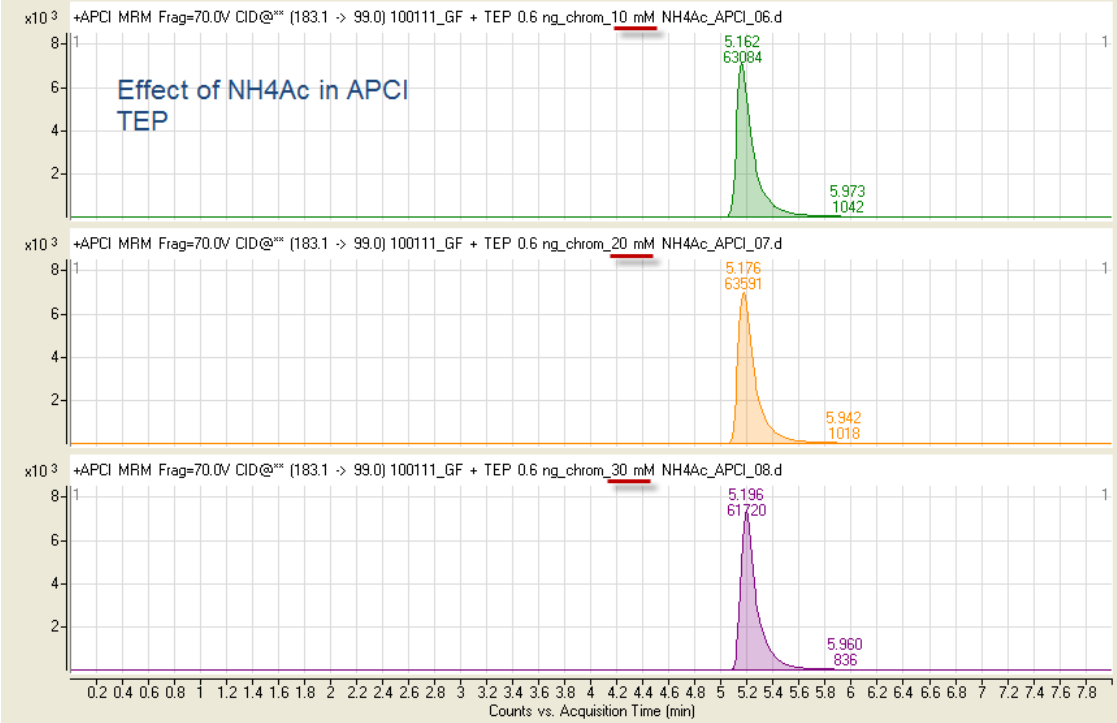

Figure 1.6g – the effect of NH4Ac concentration on TEP response in APCI

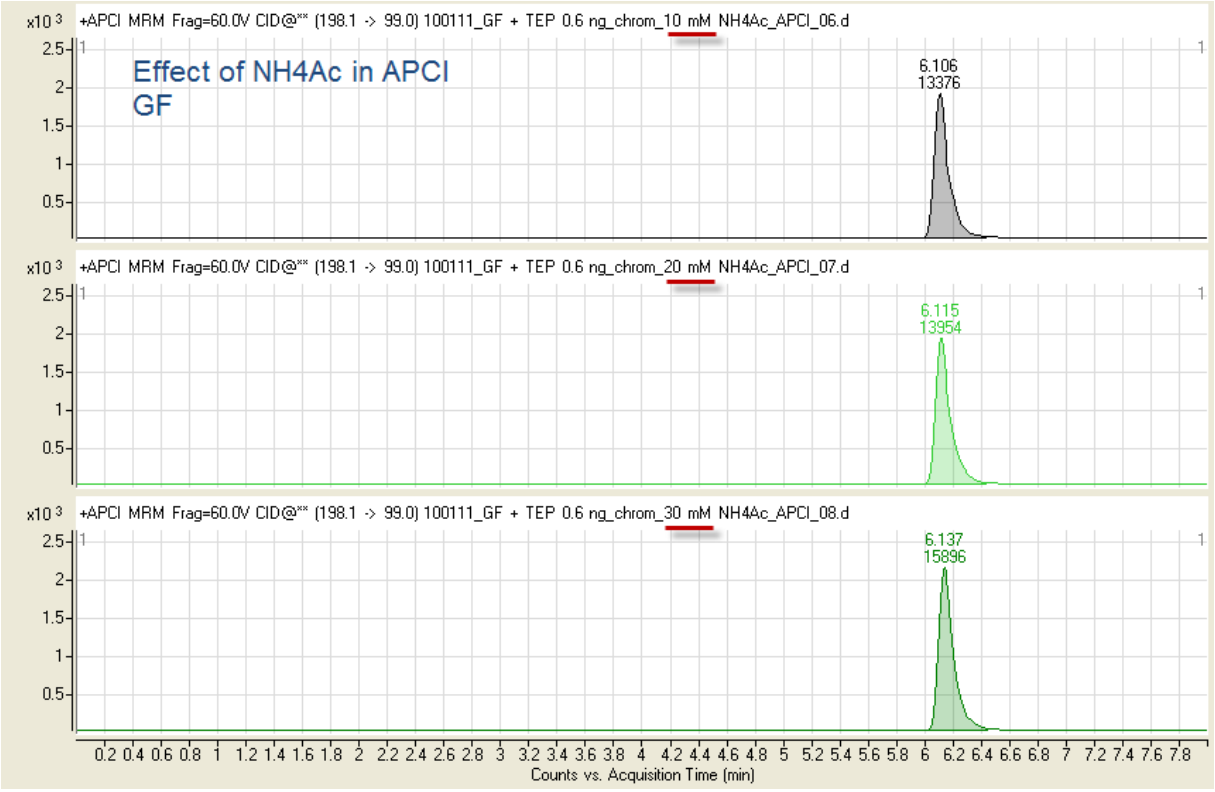

Figure 1.6h – the effect of NH4Ac concentration on GF response in APCI

The effect of having NH4Ac in the organic mobile phase (B) was investigated. Figure 1.6i shows no appreciable difference between no NH4Ac and 5mM NH4Ac in the methanol mobile phase. Therefore, it is sufficient to add NH4Ac only to the A mobile phase.

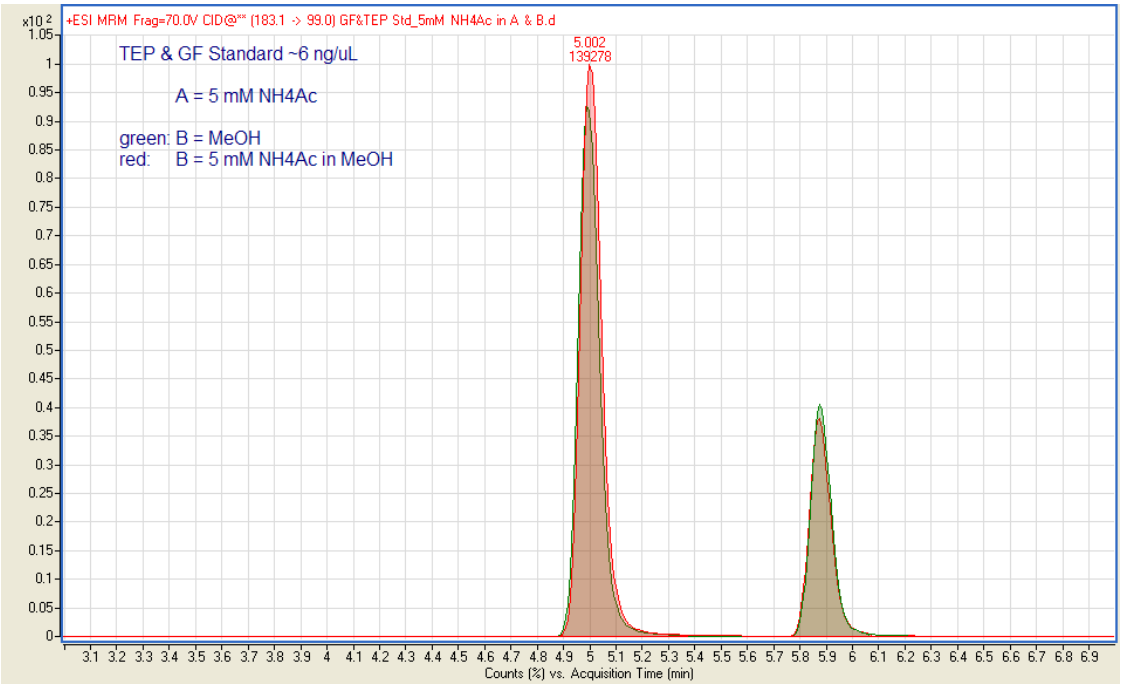

Figure 1.6i – effect of NH4Ac in methanol on TEP and GF

Methanol and acetonitrile were compared as the organic mobile phase in both APCI and ESI. Figures 1.6j & k show that TEP response was reduced in ACN by almost 50% whereas GF was reduced by more than 90%. Therefore, MeOH was used as the organic mobile phase.

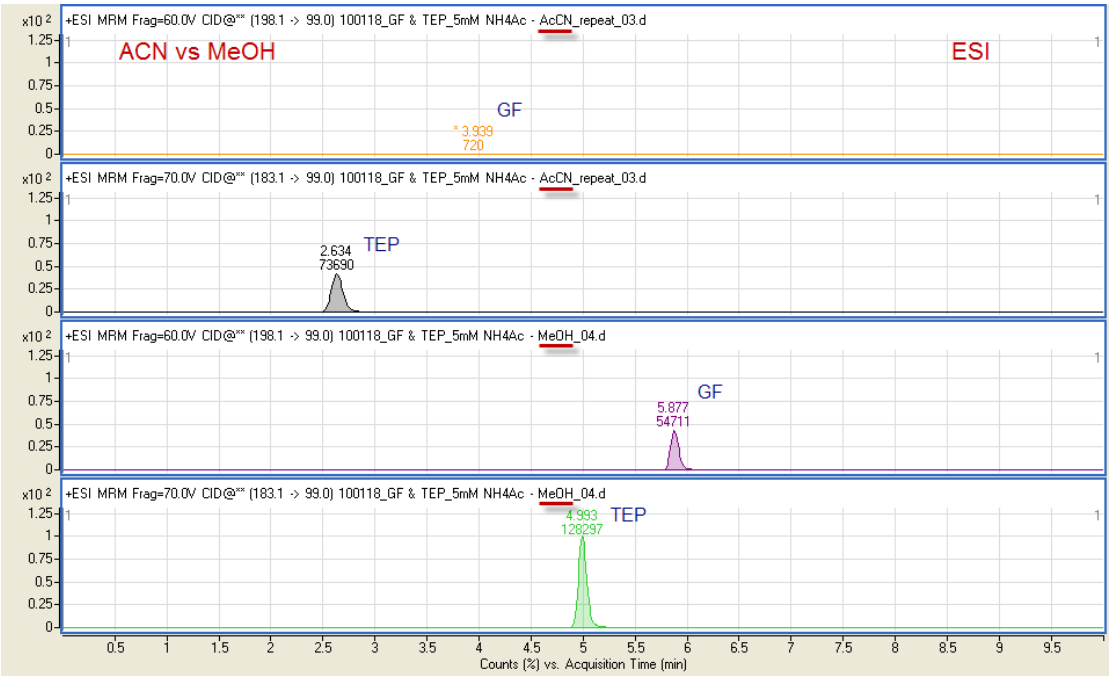

Figure 1.6j – ACN and MeOH response of TEP and GF in ESI

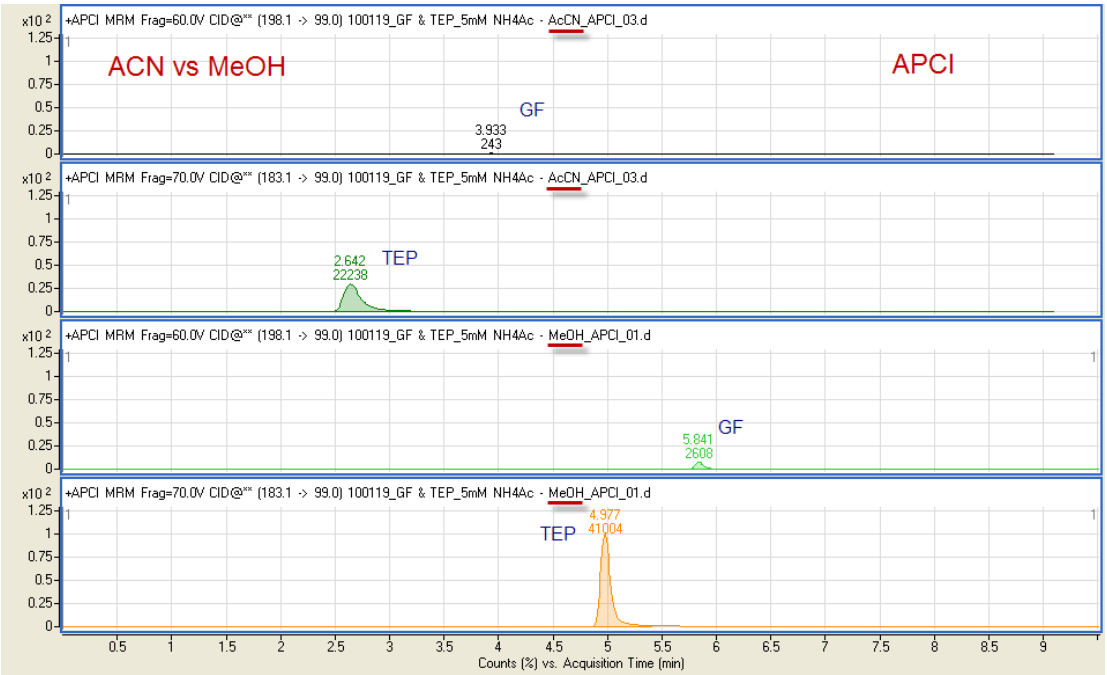

Figure 1.6k – ACN and MeOH response of TEP and GF in APCI

The LC stop time was adjusted to ensure all matrix compounds eluted before reverting to initial LC conditions. Figure 1.6l shows that both F54 and RSDL matrix components elute using the final LC conditions.

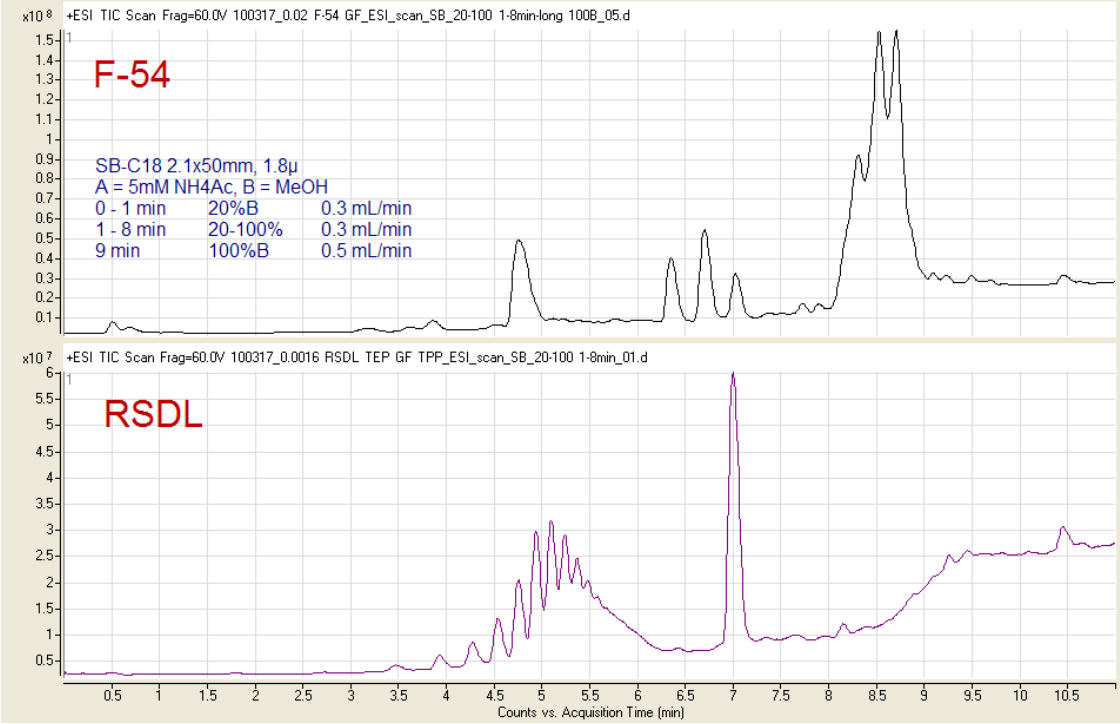

Figure 1.6l – F54 and RSDL components elution profile under final LC conditions

The effect of different column temperature was investigated. There was no appreciable difference in separation of matrix components and target analytes between 30, 40 and 50°C (data not shown). Therefore, 30°C was chosen to keep the column temp above ambient. Note the column temperature can be increased if column backpressure begins to increase due to use over time. The time segments of the MRM analysis may have to be adjusted if retention times are significantly altered.

The final MRM methods incorporate time segments that divert the LC flow to waste at the beginning and end of run. In this way, LC flow is only going into the MS system when the analytes of interest are eluting, preventing potential contamination.

Note that after performing analysis of a batch of decon experiment solutions, it is recommended to flush the column with ACN and then MeOH to ensure the matrix has been sufficiently cleaned from the system. Methods were created for that purpose for both ESI and APCI ion sources. Printouts of the method are included in Annex 1, and they are stored on the 6460 workstation in the "D:\MassHunter\Methods\!Decon Experiments" folder.

1.7 Review and optimize sampling procedure

The decon solution experiment sampling procedure used in the past was discussed with the Scientific Authority. The specific outputs/requirements of the experiment were to detect CWAs in complex decon matrices down to at least 1% of the starting concentration. Based on this, tests were performed with dilutions of the MPEG polymer used as the solvent in RSDL to determine matrix effects (ion suppression). The IDLs were used to determine levels of agent and ISTDs required to provide strong enough signal that would result in tracking of the decon solution effectiveness. A final consideration was the initial concentration of stock solutions used, as the number of personnel involved from a safety perspective is dependent on the concentration of agent in solution. A spreadsheet entitled "Decon Experiment Design - establish dilutions.xls" was developed to assist in the design of decon experiments using either diluted or neat decon solutions and agents.

The optimized sampling procedure was tested with RSDL and GF. TPP was added to the decon sample at the beginning of the experiment, and TEP was added to final dilution. Results are shown in table 1.7a. TEP and TPP recoveries look very consistent, ranging from 91.7 – 109.4%, indicating no ion suppression and no losses during sample handling.

Sample				TEP Results			GF-NH4 Results			TPP Results		
Name	Type	Level	Acq. Date-Time	RT	Area	Accuracy	RT	Area	Accuracy	RT	Area	Accuracy
ACN blank	Blank		2010/16/03 16:48	4.967	347		5.800	19		7.041	6939	
GF TEP TPP in ACN	Cal	1	2010/16/03 17:02	4.931	82854	96.5	5.776	34107	98.1	7.023	33447	104.3
ACN blank	Blank		2010/16/03 17:17	4.924	268					7.031	2628	
RSDL decon_GF_93MM11-1a	QC	1	2010/16/03 17:31	4.933	82661	96.3	5.777	239	0.7	7.022	34401	107.3
RSDL decon_GF_93MM11-1b	QC	1	2010/16/03 17:46	4.936	79185	92.3				7.026	34445	107.4
RSDL decon_GF_93MM11-1c	QC	1	2010/16/03 18:00	4.932	81374	94.8	5.788	28	0.1	7.031	35064	109.4
RSDL decon_GF_93MM11-1d	QC	1	2010/16/03 18:15	4.932	84794	98.8				7.027	33699	105.1
GF TEP TPP in ACN	Cal	1	2010/16/03 18:30	4.940	87920	102.4	5.780	35513	102.1	7.024	32225	100.5
ACN blank	Blank		2010/16/03 18:44	4.958	282					7.020	2109	
ACN blank	Blank		2010/16/03 21:09	4.929	405					7.020	890	
GF TEP TPP in ACN	Cal	1	2010/16/03 21:24	4.933	85502	99.6	5.777	34692	99.7	7.019	31084	97.0
ACN blank	Blank		2010/16/03 21:38	4.962	283					7.019	644	
RSDL decon_GF_93MM11-1a	QC	1	2010/16/03 21:53	4.929	81623	95.1	5.780	217	0.6	7.022	31936	99.6
RSDL decon_GF_93MM11-1b	QC	1	2010/16/03 22:08	4.929	78733	91.7				7.022	31666	98.8
RSDL decon_GF_93MM11-1c	QC	1	2010/16/03 22:22	4.930	80343	93.6				7.019	32207	100.5
RSDL decon_GF_93MM11-1d	QC	1	2010/16/03 22:37	4.930	83079	96.8				7.009	31705	98.9
GF TEP TPP in ACN	Cal	1	2010/16/03 22:51	4.931	87051	101.4	5.771	34806	100.1	7.019	31481	98.2
ACN blank	Blank		2010/16/03 23:06	4.933	359					7.017	563	

Table 1.7a – results of decon experiment performed with RSDL and GF after protocol optimization

Table 1.7b provides suggested concentration ranges in the final diluted solutions from a decon experiment. See section 1.8 for a discussion on matrix effects with decon solutions and appropriate final concentrations for analysis.

Ionization Mode	Compound / Solution	Concentration Range*
ESI+	TEP	0.05 – 0.2 ng/µL
	TPP	0.01 – 0.1 ng/µL
	GF	0.5 – 2 ng/µL
	RSDL	< 0.002%
	British Decon	< 0.01%
APCI+	TEP	0.75 – 5 ng/µL
	TPP	0.3 – 1 ng/µL
	GF	5 – 30 ng/µL
	RSDL	< 0.02%
	British Decon	< 0.01%

* approximate concentration range in final diluted solution for LC-MS/MS analysis

Table 1.7b - concentration ranges for decon experiments

Table 1.7c shows concentrations and volumes that were used for a decon solution experiment and the resulting concentrations in the final dilution. Note that the final GF concentration (in red) was below lowest recommended concentration listed in table 1.7b. As such, either a more concentrated solution of GF or a diluted solution of RSDL should have been used at the beginning of the experiment. This would have allowed for less severe dilutions in order to provide a higher concentration of GF in the final dilution for analysis, while still reducing the RSDL concentration to a point where ion suppression does not occur.

Solution	Solution Concentration	Volume added	Concentration in Experiment
RSDL	100%	100 µL	10%
GF	1064 ug/µL	850 µL	904 ng/µL
TPP	856 ug/µL	50 µL	43 ng/µL

Dilutions performed: 15 µL into 1000 µL, twice

Solution	Solution Concentration	Volume added	Concentration in Final Dilution
RSDL	-	-	0.0023%
GF	-	-	0.2 ng/µL
TPP	-	-	0.01 ng/µL
TEP	9.2 ng/µL	10 µL	0.09 ng/µL

Table 1.7c – example concentrations and volumes for an RSDL experiment analysed by ESI

Since the agent will most likely be deactivated by the decon solution, the precision (or error) for the detection of the agent can be estimated from precision of TEP & TPP.

Recovery of TEP and TPP should be used to gauge the success of the decon experiment. Acceptable recovery is estimated as 85% to 115%.

It should be noted that mixing of the decon and diluted solutions is critical to the success of the decon experiment. The Gilson automated liquid handler does not perform adequate mixing for all matrices, and therefore mixing by hand should be performed prior to any aliquot being withdrawn from the vial. In addition, solutions should be visually checked after mixing to ensure there is no phase separation or precipitation that could impact the final results.

Finally, it is recommended to run ACN and MeOH flushes of the system after a batch of decon experiment samples has been analysed. These methods have been created and are included at the end of the run in the worklist templates generated for this contract.

1.8 Measurement and compensation for any ion suppression/enhancement

Matrix effects are a well established phenomenon in ESI. Commonly, ions of target compounds are suppressed if components from the sample matrix elute from the LC and enter the source at the same time. Enhancement of target ion signal is also possible. In decon solution experiments, it is very important to be sure that a reduction in recovery of a CWA is due to deactivation by the decon solution and not ion suppression. Therefore much work was performed to investigate and minimize ion suppression.

Tests were initially performed with the MPEG polymer used as the solvent in RSDL, and then with actual RSDL. In order to perform ion suppression tests with an agent in RSDL, it was necessary to deactivate or quench the active ingredient in RSDL. This was done by diluting RSDL with 0.1% acetic acid. A dilution of 25 µL RSDL into 1.66 mL gave a final MPEG concentration of 1.5%. This solution was shaken and very quickly went colourless, indicating deactivation of the active ingredient. Full deactivation and/or adequate quenching through dilution was proven by injecting solutions of agent and ISTD in dilutions of this deactivated RSDL over several days, and the concentration of GF remained consistent (data not shown).

Ion suppression testing was performed using “fast chromatography” where matrix and target compounds co-eluted, as a worst case scenario. This was done by running isocratic LC with a high %MeOH. Ion suppression was also investigated using regular gradient chromatography as a best case scenario.

Both ESI and APCI sources were used. APCI tends to be less sensitive than ESI to LC mobile phase composition and also typically exhibits less matrix effects.

The LC conditions used for final evaluations of ion suppression in deactivated RSDL are shown in table 1.8a.

LC parameter	regular gradient	isocratic - fast chromatography
column	XDB-C18, 4.6x50mm, 1.8u	XDB-C18, 4.6x50mm, 1.8u
mobile phase	A = 5 mM NH4Ac, B = MeOH	A = 5 mM NH4Ac, B = MeOH
gradient	20% B for 0-1 min 20-100%B from 1-8 min stop 11 min	70% B for 0-2 min 70-100%B from 2-2.1 min stop 4 min

Table 1.8a – LC conditions for ion suppression tests

ESI does indeed show ion suppression for TEP and GF in RSDL. Figures 1.8a & b show the decrease in TEP and GF signal when dissolved in dilutions of deactivated RSDL using regular gradient chromatography and fast chromatography and ESI. Interestingly, there was less suppression of TEP and GF in fast chromatography. TEP showed a higher degree of ion suppression using gradient chromatography. As such, it would not function well as an actual internal standard (i.e. using ratios of target to ISTD to calculate final concentrations). It would, however, be a good model compound to indicate possible ion suppression in decon solutions. Dilutions of RSDL to 0.0016% do not show ion suppression using regular gradient chromatography.

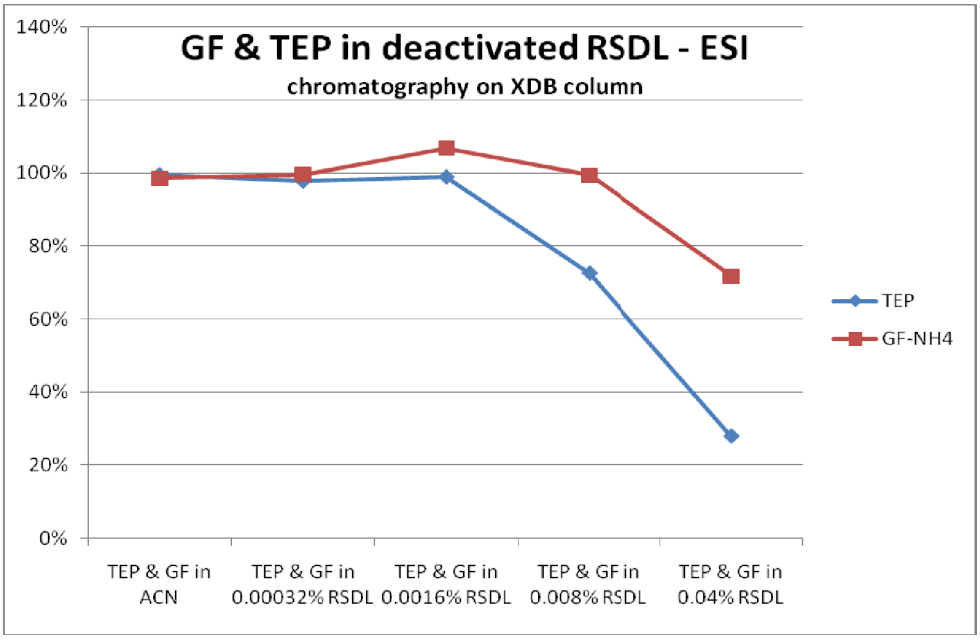

Figure 1.8a – GF and TEP in deactivated RSDL using gradient chromatography and ESI

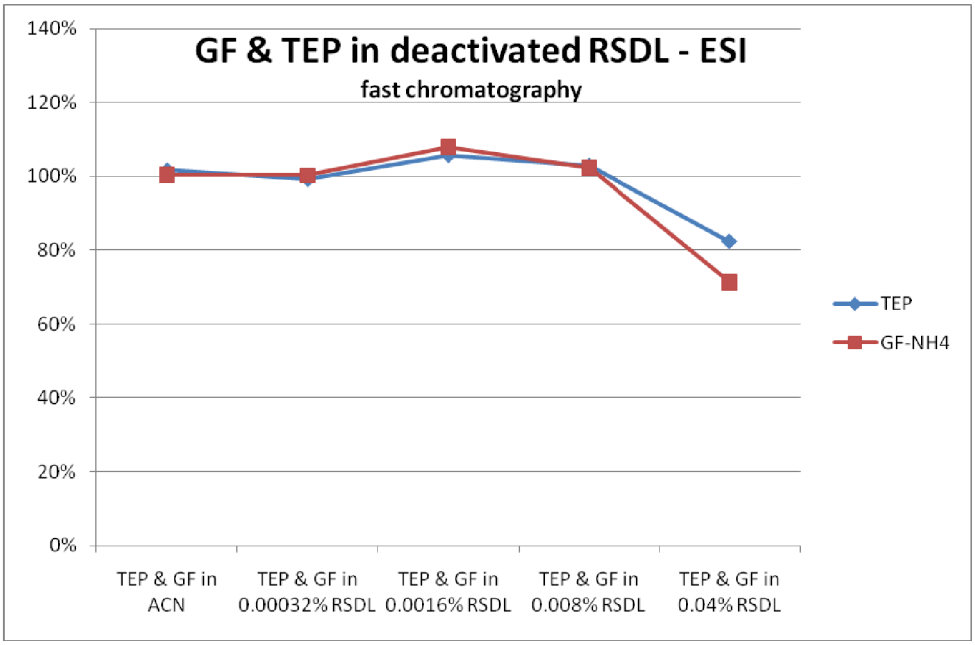

Figure 1.8b – GF and TEP in deactivated RSDL using fast chromatography and ESI

As expected, APCI shows fewer matrix effects than ESI. Figures 1.8c & d show TEP and GF signal when dissolved in dilutions of deactivated RSDL using regular gradient chromatography and fast chromatography and APCI. The signal remained relatively consistent regardless of the concentration of deactivated RSDL matrix present.

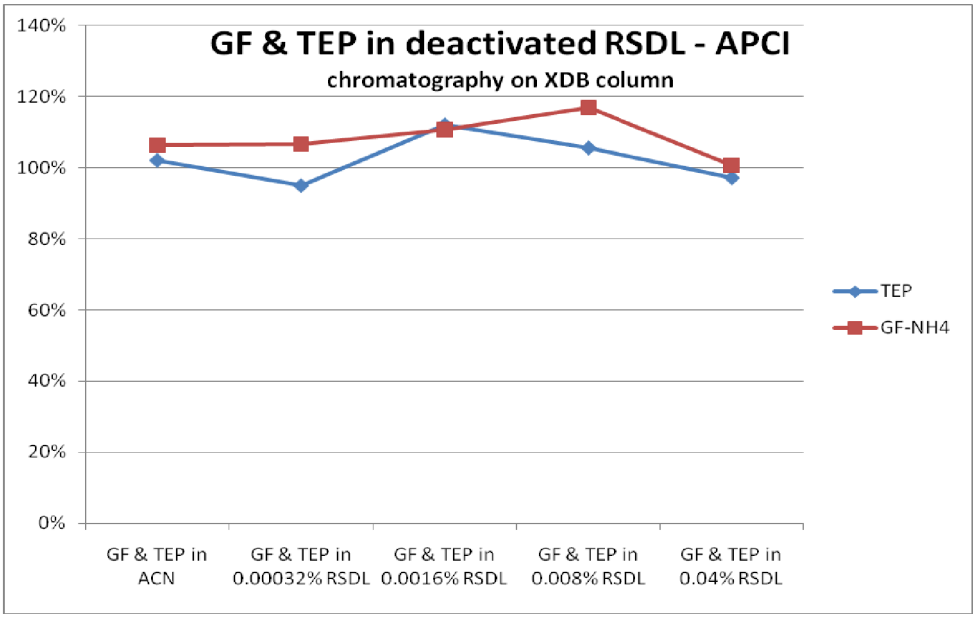

Figure 1.8c – GF and TEP in deactivated RSDL using gradient chromatography and APCI

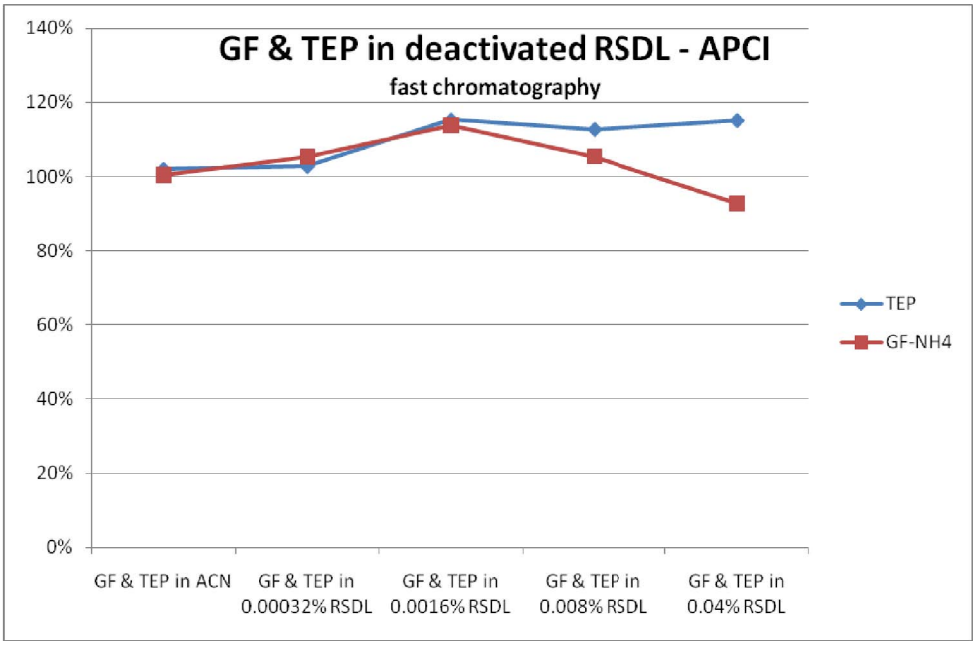

Figure 1.8d – GF and TEP in deactivated RSDL using fast chromatography and APCI

Despite the fact that APCI shows less matrix effects, the final recommendation for the MRM method is to use ESI and dilute the decon solution matrix to the point where no ion suppression is observed. There are several reasons for this decision:

- ESI is more precise than APCI (see section 1.11).
- With ESI being more sensitive than APCI, the decon solutions can be diluted to the appropriate level and target compounds still detected.
- Higher levels of dilutions required for ESI will increase the opportunity for quenching of the active ingredients in the decon solution.
- Higher levels of dilutions required for ESI will keep the LC-MS system much cleaner in the long run, resulting in more reliable data and increased instrument up-time.

The only occasion that would warrant using APCI is when high levels of a decon formulation must be run, i.e. significant dilution to levels where ESI works well is not possible. In this case, APCI can be used, however, each vial should be run in replicates of three injections due to the reduced precision.

Matrix effects were also determined for British Decon which incorporates F54. Potential ion suppression of GF and TEP in F54 matrix was investigated in ESI and APCI using fast and regular gradient chromatography. Only gradient chromatography results are shown. The actual British Decon solution includes F54 plus the active ingredient which contains sodium, which may

interfere with the ammonium adduct formation for the G agents. Therefore, a solution mimicking the British Decon solution was prepared with a compound similar to the active. This mimic solution contained an equi-molar amount of sodium, but no active decontaminant.

The results show that the F54 matrix itself does not cause ion suppression for GF and TEP. Figure 1.8e shows results for TEP and GF in F54 using gradient chromatography and ESI. At the highest concentration of F54 (0.2%), there may be a small amount of ion enhancement, but certainly no ion suppression.

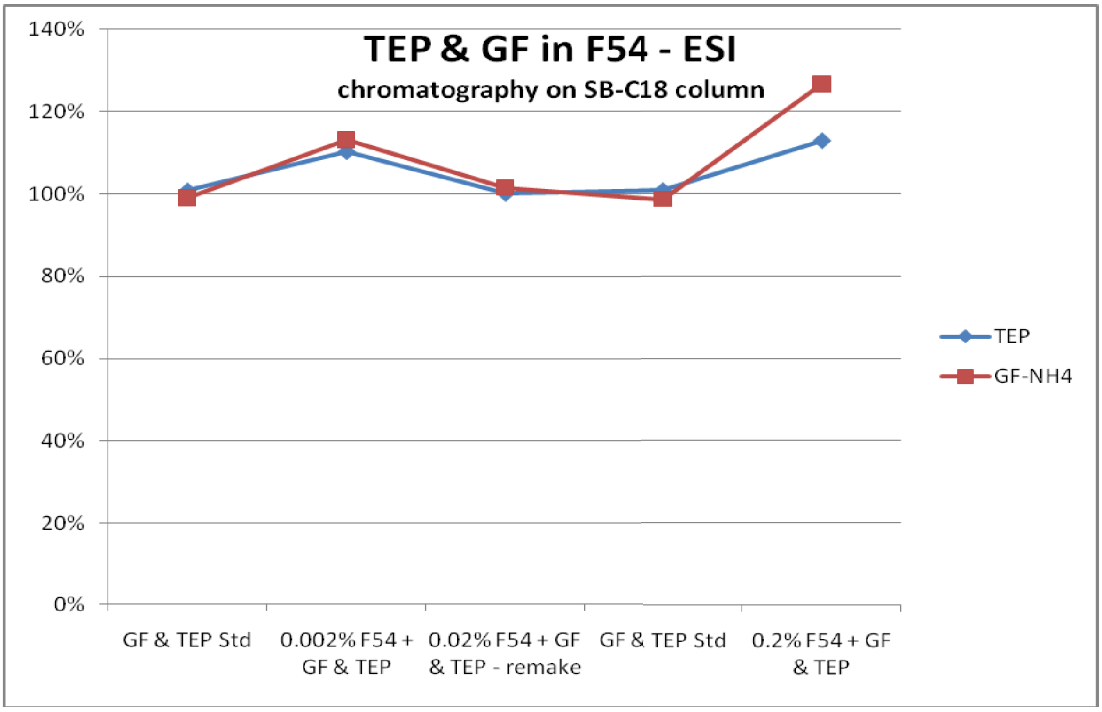

Figure 1.8e – TEP and GF in F54 dilutions by ESI

Figures 1.8f & g show that the addition of the sodium in the mimic solution has a significant ion suppression effect in both ESI and APCI. The signal for GF in the 0.2% F54 mimic solution drops to zero, while the signal for GF in the same level of F54 without sodium is unaffected. This supports the theory that increased sodium in the decon solution is creating sodium adducts for GF, thereby reducing the signal for the NH4 adduct. TEP is unaffected by the addition of sodium as it does not tend to form adducts as easily.

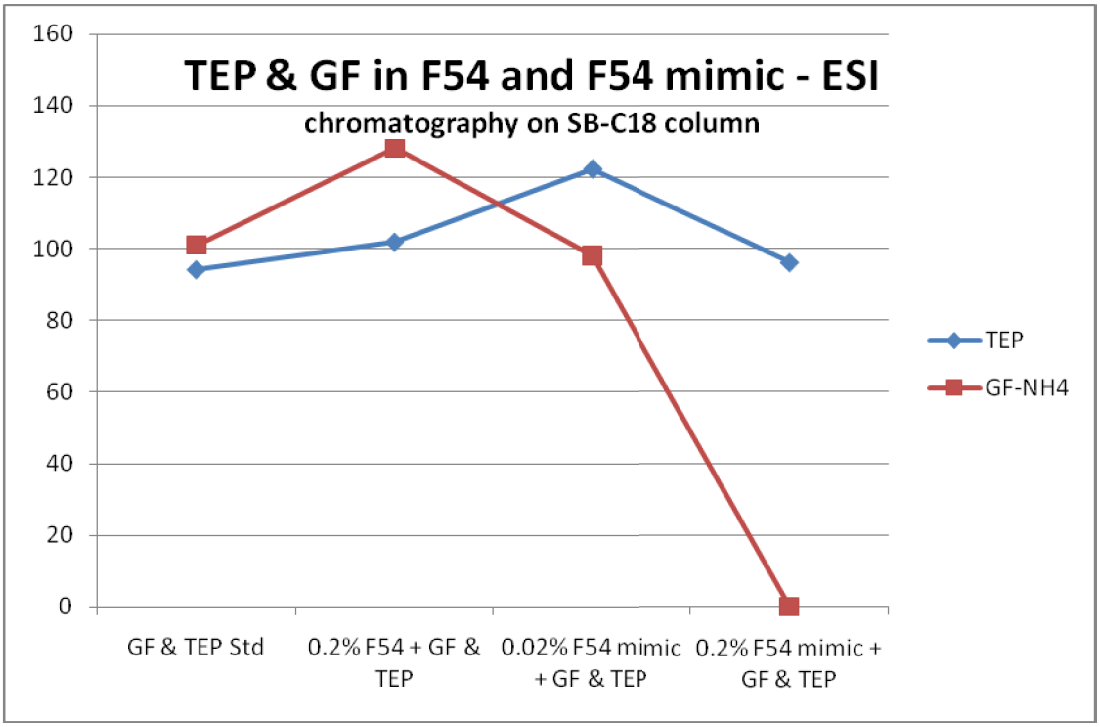

Figure 1.8f – TEP and GF in F54 and F54 mimic dilutions by ESI

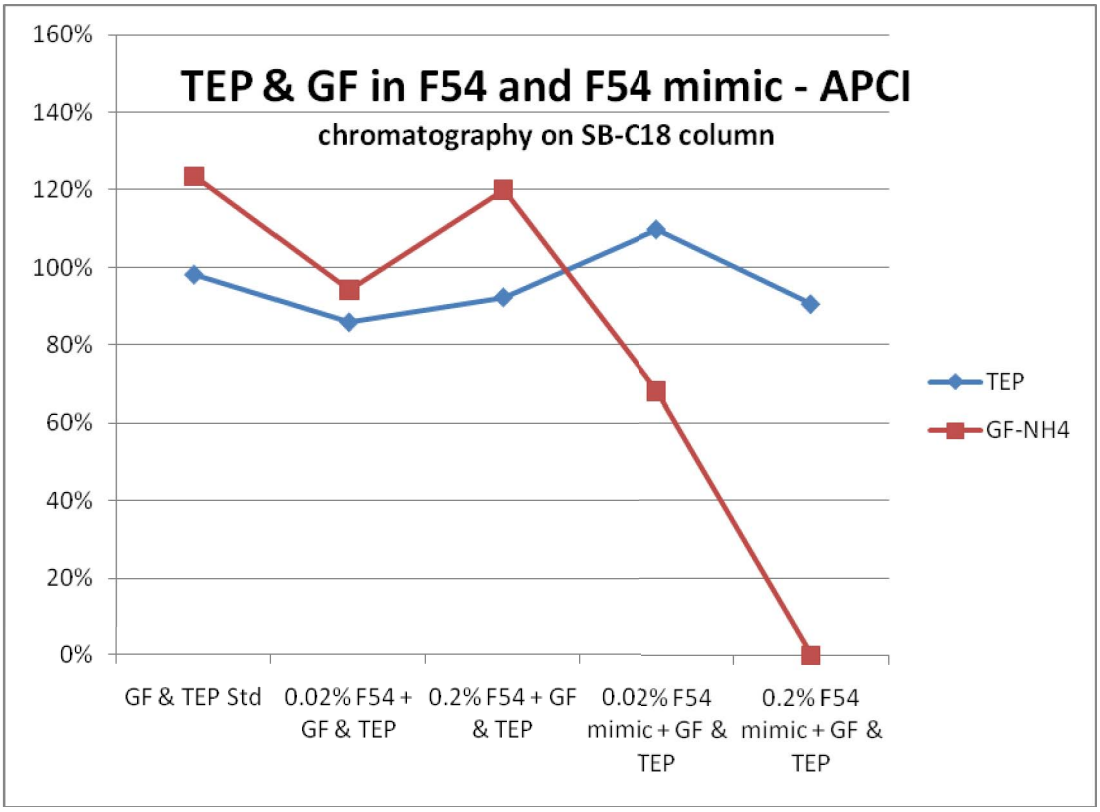

Figure 1.8g – TEP and GF in F54 and F54 mimic dilutions by APCI

Adding a higher concentration of NH4Ac in the mobile phase decreased the amount of ion suppression for GF in the F54 mimic solution (data not shown). The drawback of this approach is that the overall sensitivity in ESI drops significantly with increased NH4Ac concentration (see section 1.6).

Therefore, it is very important to properly dilute the British Decon (F54 with Na containing active ingredient) solutions to avoid ion suppression. In this case, unlike RSDL, TEP will not be a good indicator of ion suppression and therefore proper dilution is critical.

The recommended final concentration of British Decon (i.e. in the final dilution used for analysis) is 0.01%.

1.9 Determine the linearity of the CWA calibration and IDLs

Linearity was determined using ESI and APCI for GF, and using ESI for TEP and TPP. The calibration plots indicate that response is linear with R^2 values of 0.99 or better. The origin was ignored, and a weighting of 1/x was used to better fit to the low concentration standards. In some cases (GF using APCI and TPP using ESI), a quadratic fit produced a better calibration curve. Table 1.9a indicates the range of amounts injected on column, R^2 values and fit method.

Compound	Ionization Mode	Amount Injected (ng on-column)	R^2	Fit Method
GF	APCI	0.001 – 3	0.9994	quadratic
			0.9992	linear
	ESI		0.9996	linear
TEP	ESI	0.0005 – 1.5	0.9999	linear
TPP	ESI	0.0006 – 2	0.9999	quadratic
			0.9940	linear

Table 1.9a – linearity for GF, TEP and TPP

Graphic representations of typical calibrations curves are shown in figures 1.9a –f .

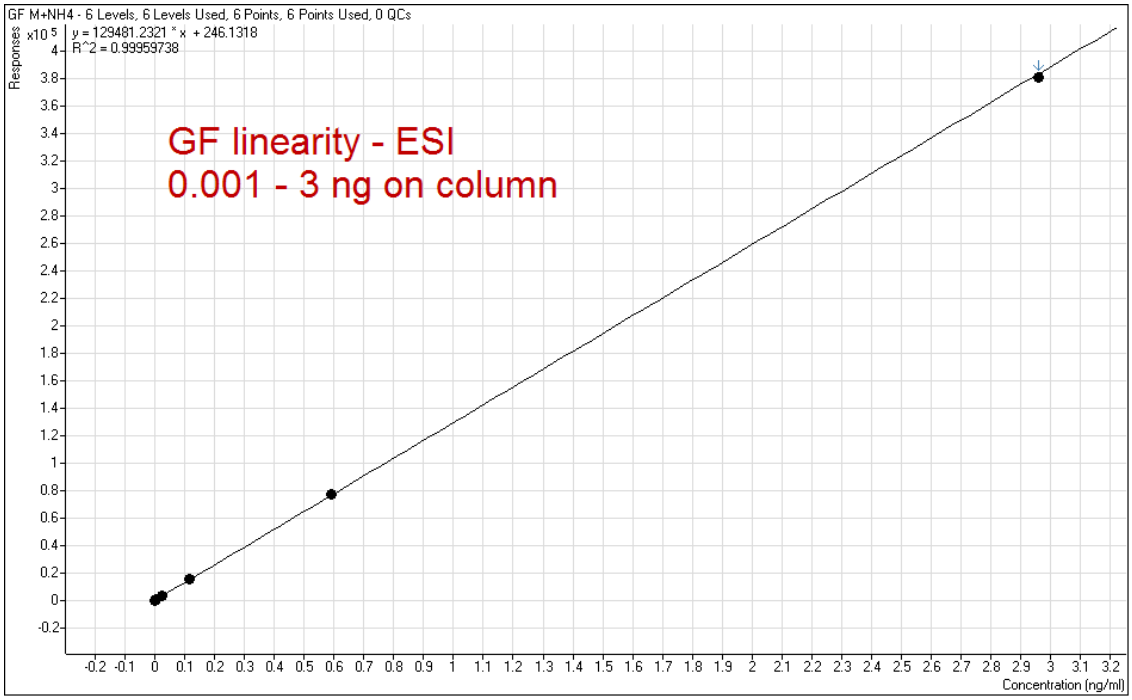

Figure 1.9a – linear fit for GF by ESI

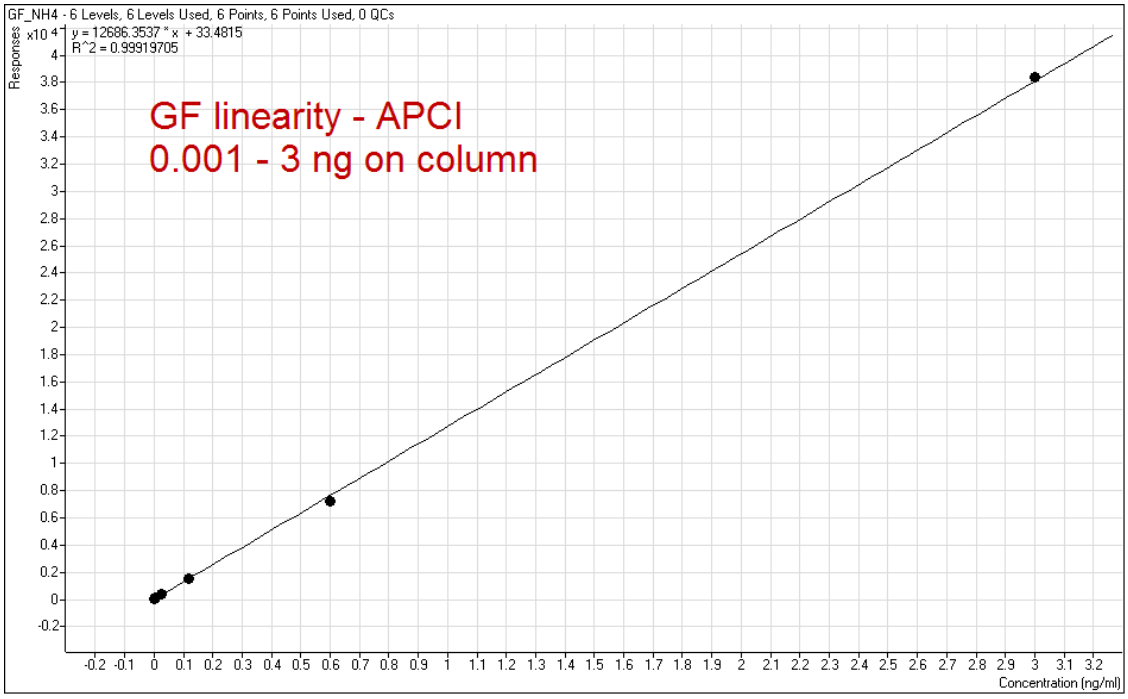

Figure 1.9b – linear fit for GF by APCI

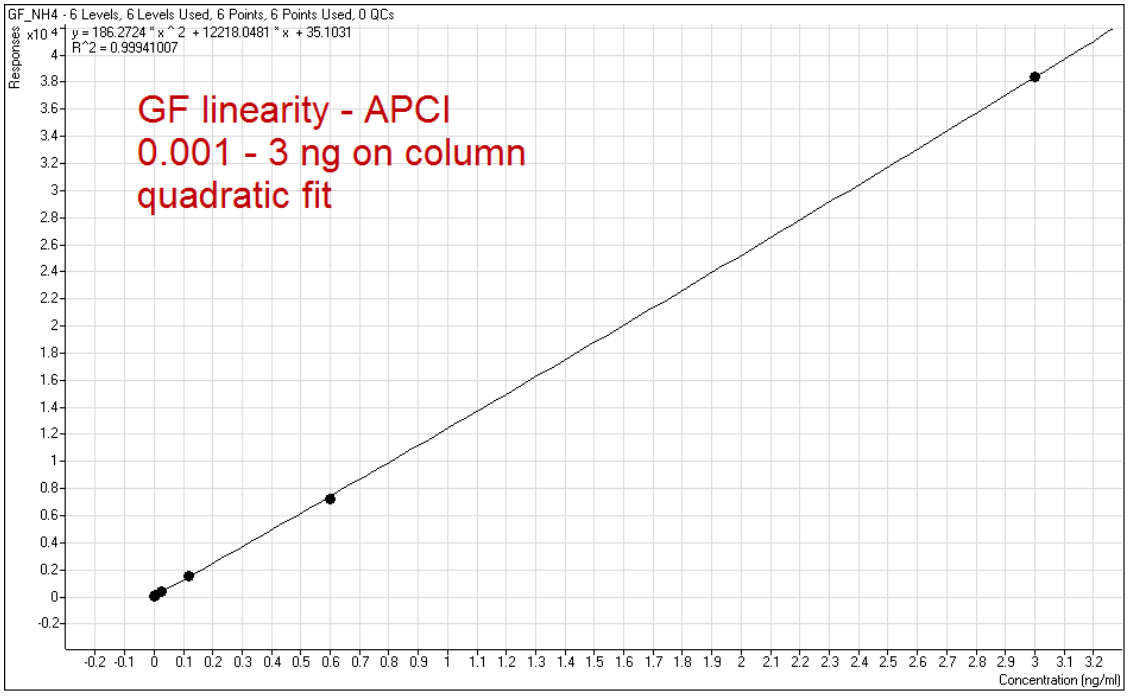

Figure 1.9c – quadratic fit for GF by APCI

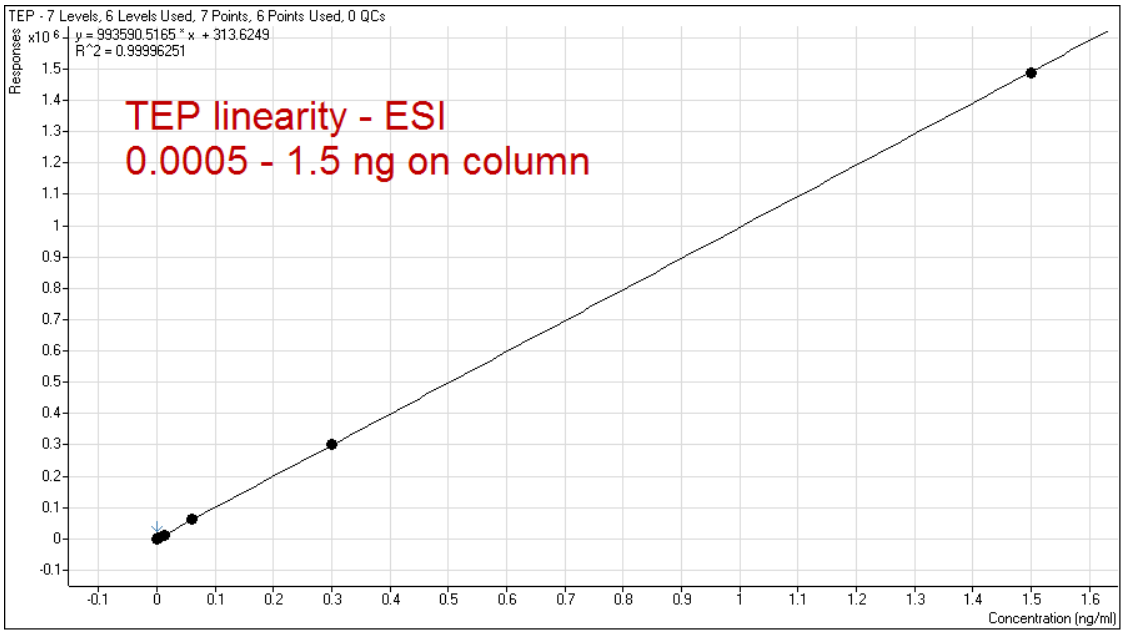

Figure 1.9d – linear fit for TEP by ESI

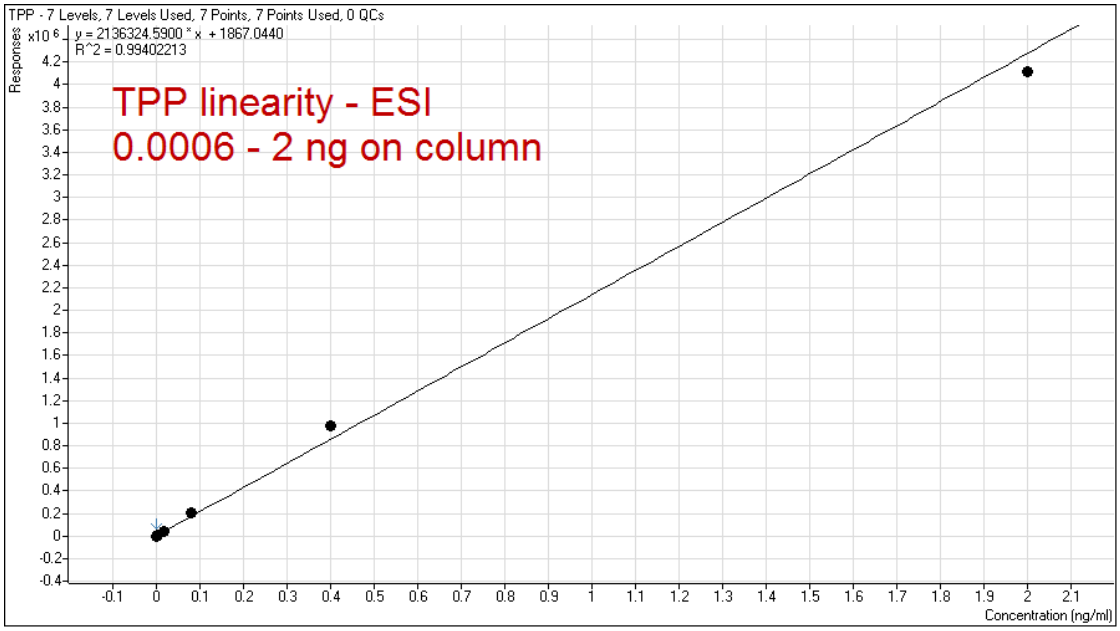

Figure 1.9e – linear fit for TPP by ESI

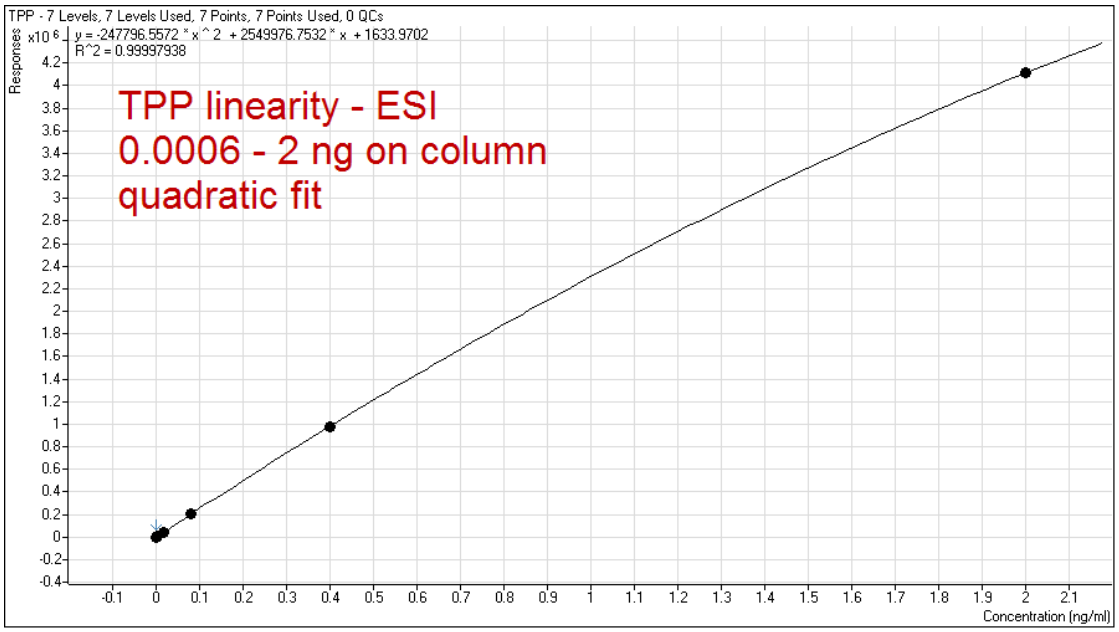

Figure 1.9f – quadratic fit for TPP by ESI

The Instrument Detection Limit (IDL) was determined for GF by ESI and APCI. The absolute signal in APCI is less than that for ESI. While the quantifying transition can be seen down to 0.001 ng on column in ESI, and 0.005 ng on column by APCI, the IDL must take into account the signal of the qualifying transition. As such, the estimated on column IDL for GF is 0.008 ng by ESI and 0.023 ng by APCI. Figures 1.9g & h show the [M+NH4]+ (top) and [M+H]+ (bottom) MRM traces for GF by ESI and APCI at 0.023 ng injected on column.

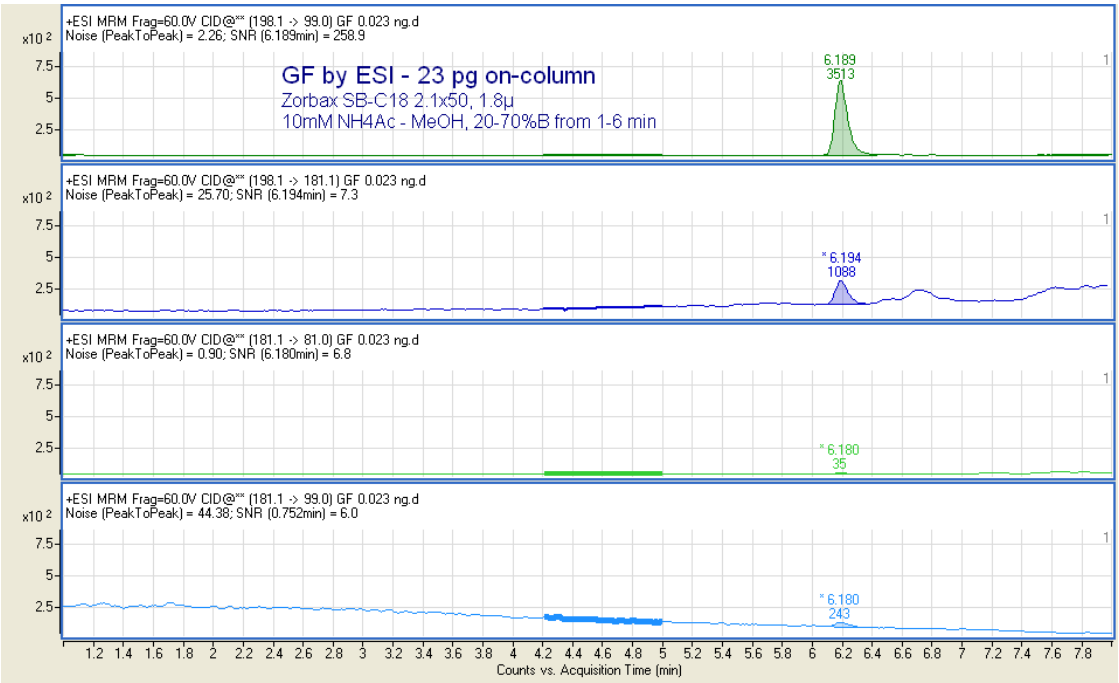

Figure 1.9g – 0.023 ng GF on column by ESI

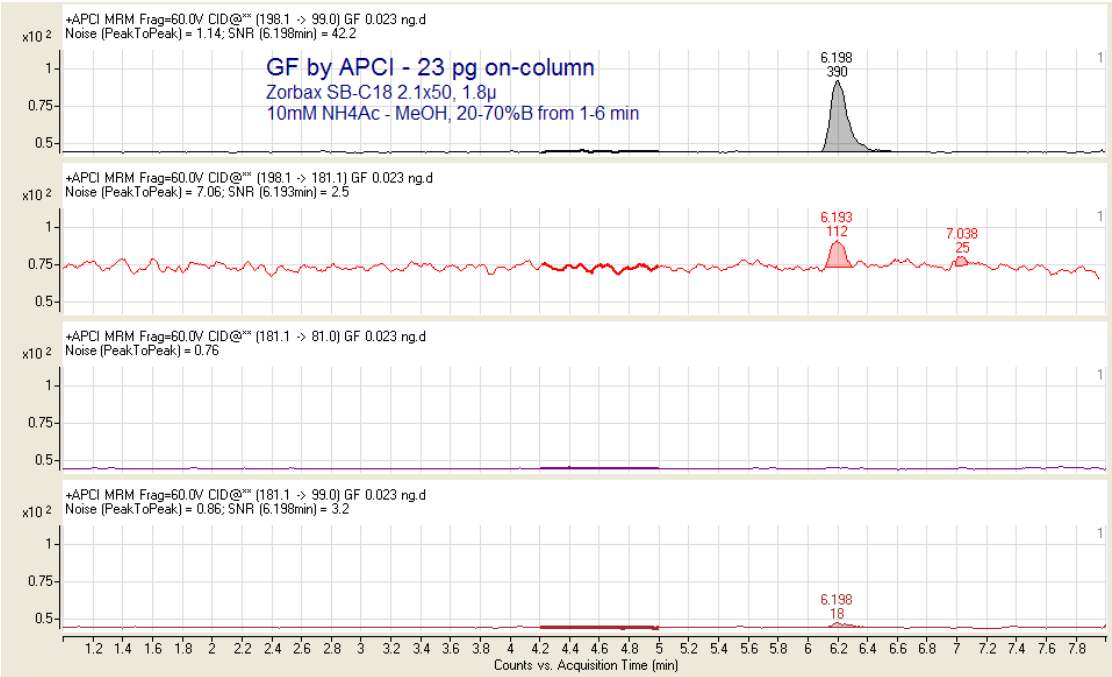

Figure 1.9h – 0.023 ng GF on column by APCI

It is interesting to note that the alkyl phosphate compounds show higher sensitivity than the CWA GF. Also, sensitivity increases as the length of the alkyl group increases, i.e. TEP < TPP < TBP. While IDLs are not strictly required for ISTDs, the estimated IDL by ESI is 0.0003 ng (or 0.3 pg) for TEP and 0.0002 ng (or 0.2 pg) for TPP. Figures 1.9i and j show TEP and TPP at 0.13 pg injected, as well as solvent blank. Note that the phosphate compounds show some carry over and are therefore present in blank injections. As such, the IDLs for TEP and TPP are blank limited (e.g. approximately 3x the level in the blank).

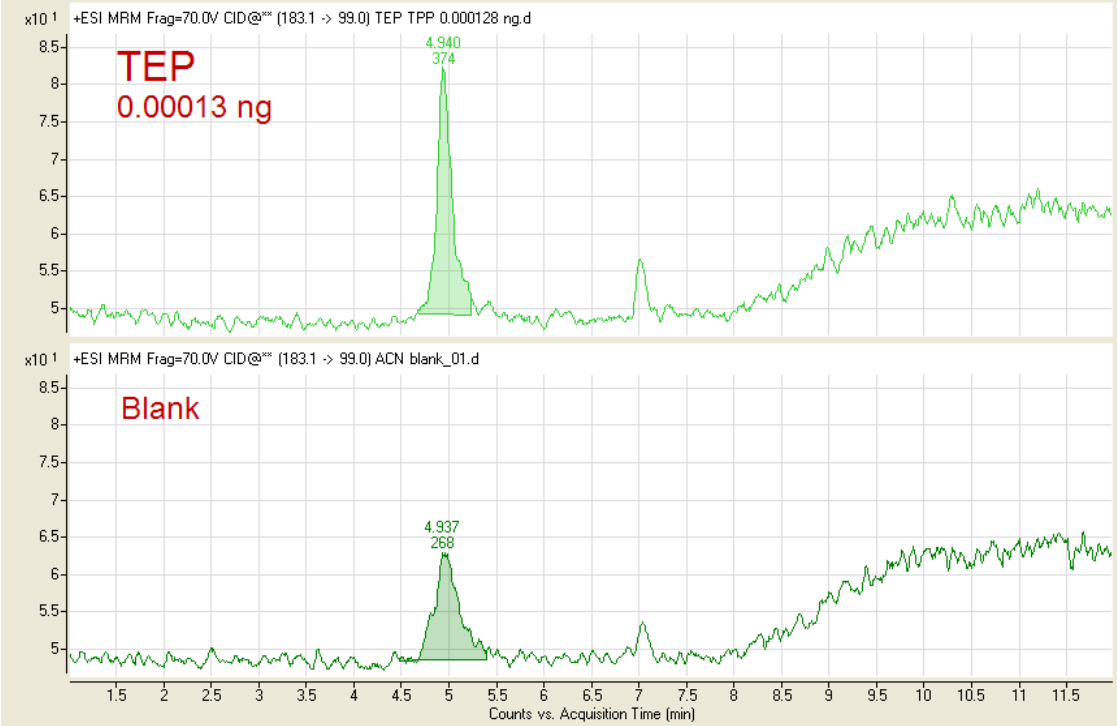

Figure 1.9i – 0.00013 ng TEP on column by ESI

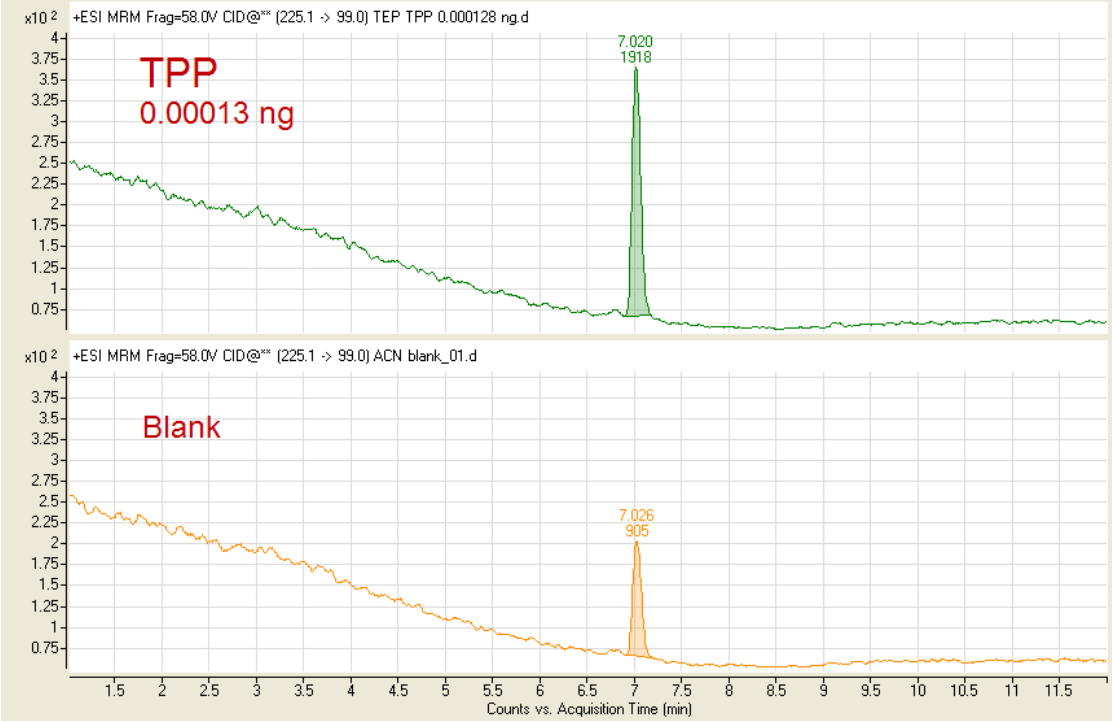

Figure 1.9j – 0.00013 ng TPP on column by ESI

1.10 Measurement of carryover

Carryover was measured in the final MRM method by injecting a high concentration standard followed by a solvent (ACN) blank. Results for GF, TEP, TPP and TBP are shown in figures 1.10a – d.

GF did not show any detectable carry over. The alkyl phosphates show some carry over, and the amount increases as the alkyl chain increases (i.e. TEP < TPP < TBP). The amount of carry over for TEP and TPP were less than 1%, which is an acceptable level for the requirements of the decon experiments.

Carry over reduction functions available in MassHunter software were used in an attempt to reduce the level of carry over for TBP. A slight reduction was noted, however the level of carry over for TBP remained over 1% which is higher than desired. For this reason, and the fact that TBP elutes much later than the CWAs tested, TBP was not chosen as a suitable ISTD or surrogate compound.

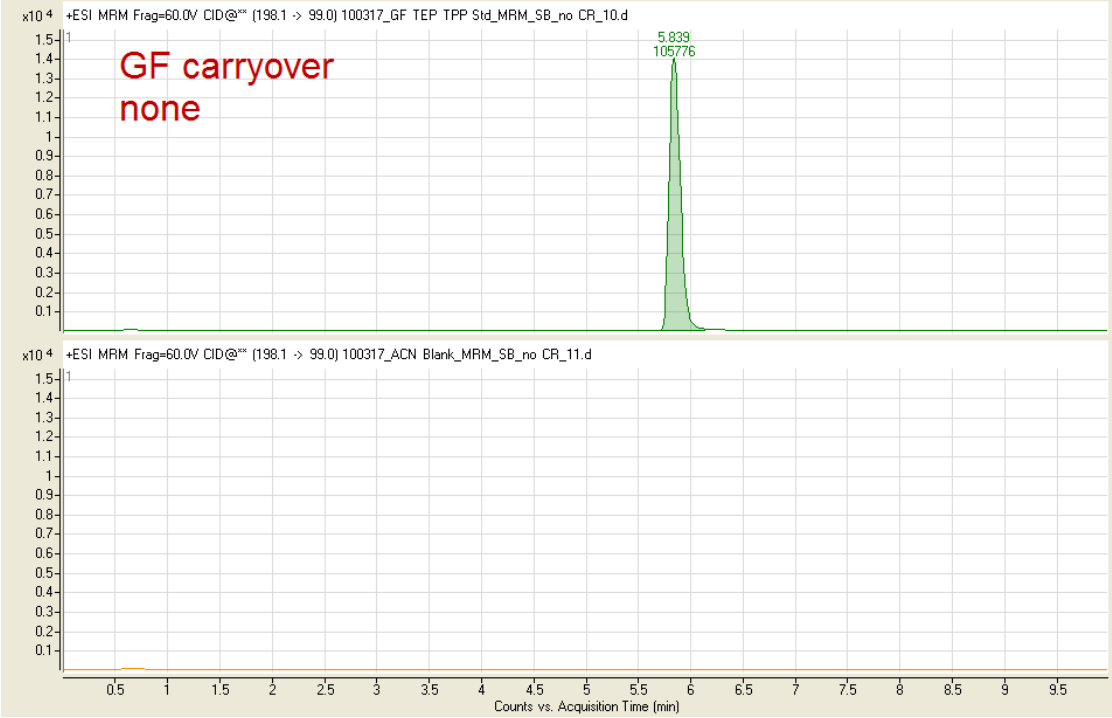

Figure 1.10a – GF carry over

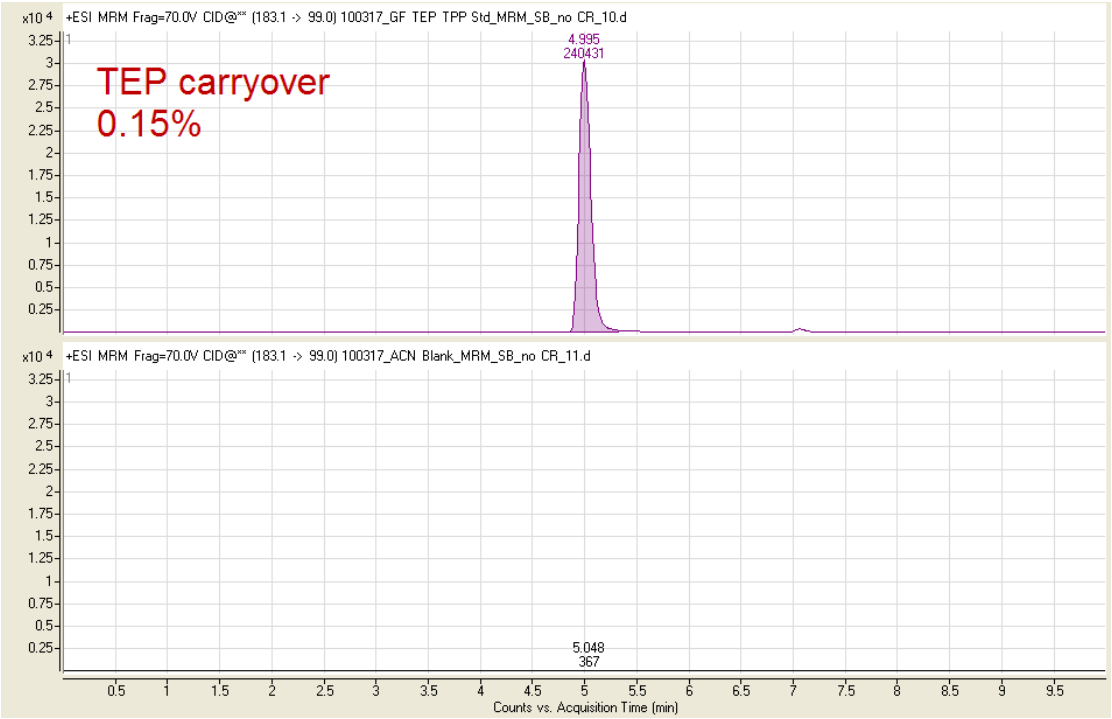

Figure 1.10b – TEP carry over

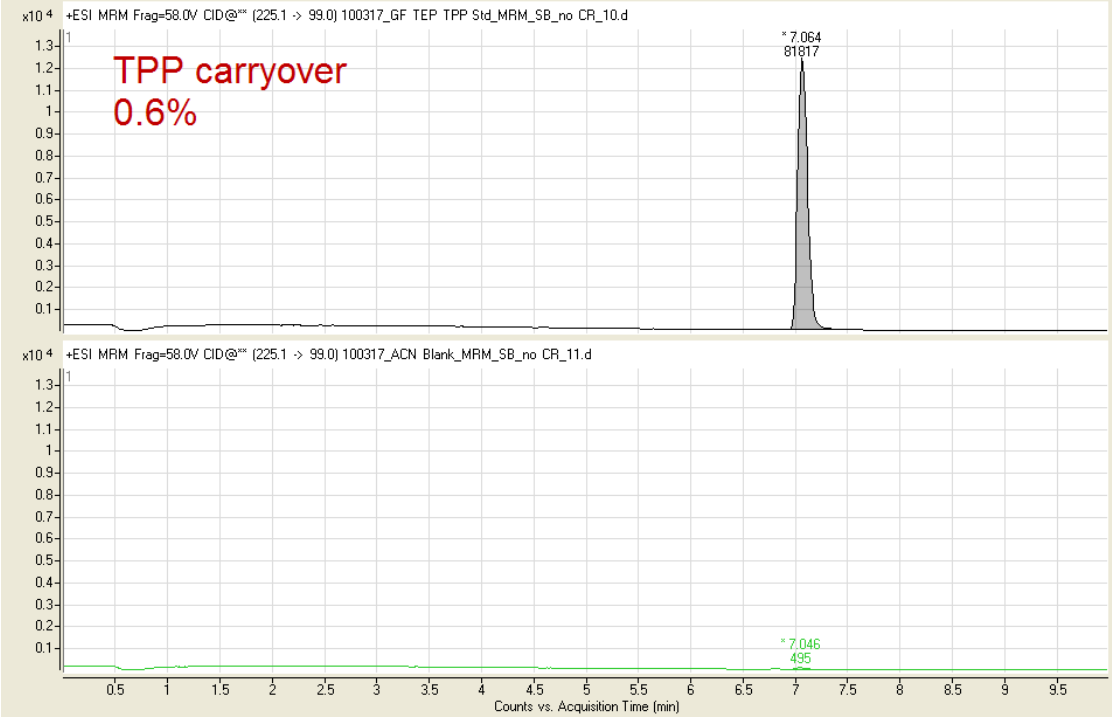

Figure 1.10c – TPP carry over

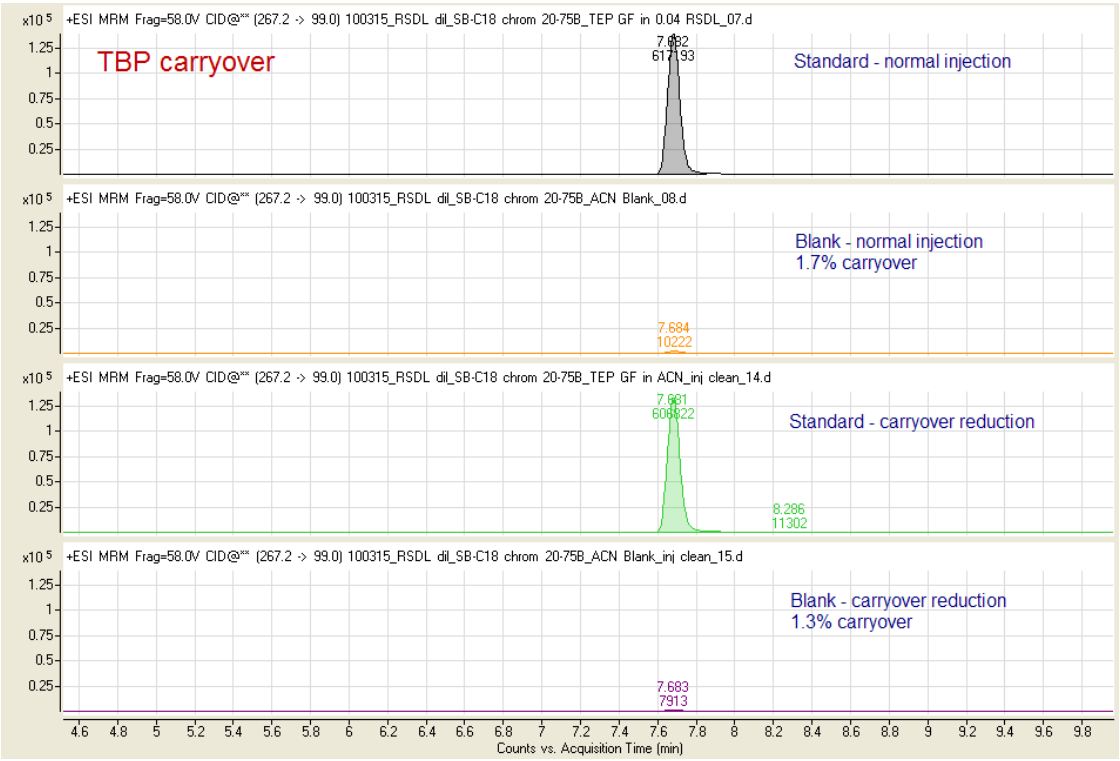

Figure 1.10d – TBP carry over, with and without carry over reduction

1.11 Measurement of instrument precision

The instrument precision was measured in both ESI and APCI. Tables 1.11a & b show the results of testing. ESI is more precise than APCI, with %RSDs of approximately 1% or less and 5%, respectively.

Name	Acq. Date-Time	TEP Area	GF-NH4 Area
GF & TEP Std	2010/1/12 17:11	54266	23248
GF & TEP Std	2010/1/12 17:25	54343	22844
GF & TEP Std	2010/1/12 17:38	54955	22845
GF & TEP Std	2010/1/12 17:51	54686	22897
GF & TEP Std	2010/1/12 18:05	54587	22689
GF & TEP Std	2010/1/12 18:18	54733	22513
GF & TEP Std	2010/1/12 18:32	54840	22611
GF & TEP Std	2010/1/12 18:45	54419	22563
	average	54603.6	22776.2
	std dev	244.9	237.9
	%RSD	0.4%	1.0%

Table 1.11a – replicate injections of the same vial of TEP & GF in ACN by ESI

Name	Acq. Date-Time	TEP Area	GF-NH4 Area
TEP & GF in AcCN	2010/3/8 17:24	7184	3281
TEP & GF in AcCN	2010/3/8 17:36	7036	3272
TEP & GF in AcCN	2010/3/8 17:48	7246	3331
TEP & GF in AcCN	2010/3/8 18:00	7282	3479
TEP & GF in AcCN	2010/3/8 18:12	7624	3578
TEP & GF in AcCN	2010/3/8 18:24	8033	3703
TEP & GF in AcCN	2010/3/8 18:36	7600	3585
TEP & GF in AcCN	2010/3/8 18:48	7634	3867
TEP & GF in AcCN	2010/3/8 19:00	7989	3641
TEP & GF in AcCN	2010/3/8 19:12	7901	3622
	average	7552.9	3535.7
	std dev	353.8	194.2
	%RSD	4.7%	5.5%

Table 1.11b – replicate injections of the same vial of TEP & GF in ACN by APCI

Different LC flow-rates were tested (0.3 and 1 mL/min) with APCI in an attempt to improve the sensitivity and precision with APCI, however the results were similar between both flow-rates (data not shown).

1.12 Determination of sample recovery

Recovery usually refers to the amount of target compound remaining after some sample preparation steps are performed, for example, extraction, cleanup steps, concentration, etc. The method developed in this contract involves limited sample preparation steps as the only step is dilution. It is still useful, however, to ensure that the level of target compound being spiked into the decon solutions is quantitatively "recovered" when analysis of the dilutions is performed.

Recovery of CWA and alkyl phosphates in decon experiments were determined by preparing solutions in solvent (ACN) at the same concentration as used for the decon solutions. These were then used as the 100% calibrator for the MRM analysis of the compounds in decon solutions, calculated using ESTD.

At the appropriate levels of dilution where no ion suppression is found, GF and TEP were quantitatively recovered.

A decon experiment was performed using GF in RSDL, spiking TPP at the time of sample preparation (surrogate) and TEP into the final dilution vial just prior to instrumental analysis (similar to an ISTD). Samples of the decon solution were withdrawn at various times to trace effectiveness of the decon solution. The results of this experiment were calculated using ESTD and are shown in table 1.12a. The results indicate that RSDL was effective in deactivating GF as GF was only detected in the first sample, and then only at 0.6%. TEP and TPP recoveries averaged 94 and 99%, respectively, and ranged between 91.7 – 100.5%.

Sample	TEP Results		GF-NH4 Results		TPP Results	
Name	Area	Recovery	Area	Recovery	Area	Recovery
RSDL decon_GF_93MM11-1a	81623	95.1%	217	0.6%	31936	99.6%
RSDL decon_GF_93MM11-1b	78733	91.7%			31666	98.8%
RSDL decon_GF_93MM11-1c	80343	93.6%			32207	100.5%
RSDL decon_GF_93MM11-1d	83079	96.8%			31705	98.9%
	average	94.3%		0.6%		99.4%
	min	91.7%		0.6%		98.8%
	max	96.8%		0.6%		100.5%

Table 1.12a – recovery of GF, TEP and TPP in an RSDL decon experiment

When deactivated RSDL was used as the decon solution, the agent GF was also quantitatively recovered (98.9 – 105.2%) in dilutions where there was no ion suppression.

1.13 Measurement of precision for sample replicates

To estimate precision (repeatability), solutions generated in a decon experiment using RSDL with GF, TEP and TPP (as described in 1.12) were analysed by LC-ESI-QQQ and then analysis for the entire batch was repeated on the same day. The peak areas for the three compounds in solvent as well as decon solutions are presented in table 1.13a. The values for %RSD (precision) can be considered worse case as they include standards and diluted decon solutions. The results show that retention times were very reproducible, with %RSDs between 0.07 and 0.09%. Precision for TEP, GF and TPP response ranged between 1.7 and 4.1%.

Sample	TEP Results		GF-NH4 Results		TPP Results	
Name	RT	Resp.	RT	Resp.	RT	Resp.
GF TEP TPP in ACN	4.931	82854	5.776	34107	7.023	33447
RSDL decon_GF_93MM11-1a	4.933	82661	5.777		7.022	34401
RSDL decon_GF_93MM11-1b	4.936	79185			7.026	34445
RSDL decon_GF_93MM11-1c	4.932	81374	5.788		7.031	35064
RSDL decon_GF_93MM11-1d	4.932	84794			7.027	33699
GF TEP TPP in ACN	4.940	87920	5.780	35513	7.024	32225
GF TEP TPP in ACN	4.933	85502	5.777	34692	7.019	31084
RSDL decon_GF_93MM11-1a	4.929	81623	5.780		7.022	31936
RSDL decon_GF_93MM11-1b	4.929	78733			7.022	31666
RSDL decon_GF_93MM11-1c	4.930	80343			7.019	32207
RSDL decon_GF_93MM11-1d	4.930	83079			7.009	31705
GF TEP TPP in ACN	4.931	87051	5.771	34806	7.019	31481
n	12	12	7	4	12	12
average	4.932	82926	5.778	34779	7.022	32780
std dev	0.0033	2932	0.0052	577	0.0054	1356
%RSD	0.07%	3.54%	0.09%	1.66%	0.08%	4.14%

Table 1.13a – single day precision for TEP, GF and TPP

1.14 Measurement of day-to-day precision

To estimate day-to-day precision (reproducibility), a solution of TEP and GF in 0.0016% deactivated RSDL was analysed on three different days and the absolute areas were compared. This represents a worst case estimate of precision, as these are raw area counts, not a calculated amount as compared to a standard analysed on the same day. What's more, two different columns* were used between these two days. The results, shown in table 1.14a, indicate that the day-to-day precision for TEP and GF was 8% and 11%, respectively.

* columns used:

- March 10 & 11 - Eclipse XDB-C18 column (4.6x50mm, 1.8µ, 0.5 mL/min)
- March 15 - SB-C18 column (2.1x50mm, 1.8µ, 0.3 mL/min)

The GF to TEP ratio, also shown in table 1.14a, is more precise, indicating that should it be required, TEP could be used as an internal standard to help compensate for injection-to-injection and day-to-day differences.

		TEP	GF-NH4	GF-NH4 / TEP
Name	Acq. Date-Time	Area	Area	ratio
TEP & GF in 0.0016% RSDL	2010/03/10 10:58	529242	185028	0.350
TEP & GF in 0.0016% RSDL	2010/03/11 16:43	451548	149433	0.331
TEP & GF in 0.0016% RSDL	2010/03/15 9:44	528904	188379	0.356
TEP & GF in 0.0016% RSDL	2010/03/15 14:45	541409	192860	0.356
	average	512776	178925	0.348
	standard deviation	41231	19921	0.012
	%RSD	8.04%	11.13%	3.43%

Table 1.14a – day-to-day precision for TEG and GF in deactivated RSDL dilution

This degree of reproducibility shows that the method is suitable for decon solution experiments.

1.15 Measurement of accuracy

For the purposes of decon experiments, accuracy has the same meaning as recovery. See section 1.12 for the results of recovery.

1.16 Adaption of the method for the 6130 MS single quad where possible

As can be seen in the discussion of ion suppression in section 1.8, decon experiment solutions must be diluted significantly before analyzing by a "dilute-and-shoot" procedure on LC-MS. Since the ion suppression observed is an ion source phenomenon, and the 6130 single quad MS uses the same or similar source designs, the same degree of ion suppression can be expected to be seen on that instrument. The 6130, however, is much less sensitive and specific than the 6460 triple quad MS. It is doubtful, therefore, that at the levels of dilution required to eliminate ion suppression, the 6130 single quad would be able to detect CWAs at levels required to follow their deactivation by a decon solution. This was discussed with the Scientific Authority, and given the probable lack of success, it was decided not to pursue this task.

1.17 Preparation of templates for work lists, methods and reports for Agilent MassHunter and ChemStation software

No templates for ChemStation were created as it was decided not to proceed with work on the 6130 single quad, which uses ChemStation.

Templates were prepared for MassHunter work lists, methods and batch table layouts for reporting. Electronic versions were left on the 6460 workstation, as shown in figures 1.17a – d.

Acquisition methods were created for the agents GD and GF by ESI and APCI. All methods contain MRM transitions for TEP and TPP which can be used as surrogates / internal standards. System flush methods for both ESI and APCI were also created. Hardcopies of the acquisition methods are provided in Annex 1.

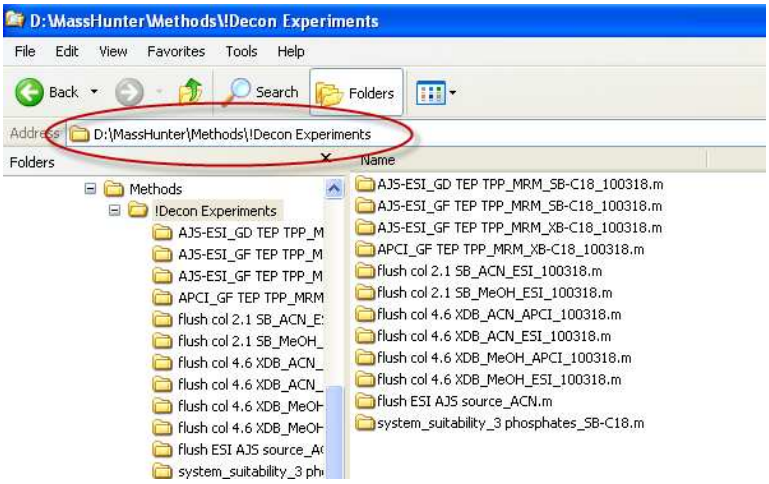

Figure 1.17a – acquisition methods in D:\MassHunter\Methods\!Decon Experiments folder

Various methods for use in Qualitative Analysis were created and are available to assist with visually processing data from full scan or MRM runs (Figure 1.17b)

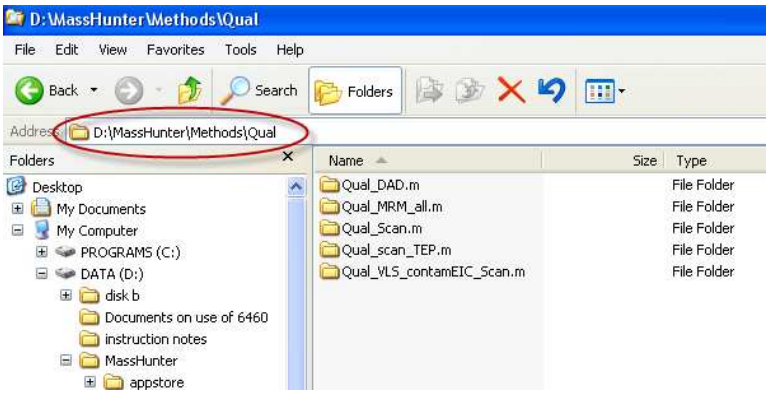

Figure 1.17b – qualitative analysis methods in D:\MassHunter\Methods\Qual folder

Various methods for processing batches of samples in Quantitative Analysis were created (Figure 1.17c). There are methods available for using ESTD and ISTD with one or five calibration levels.

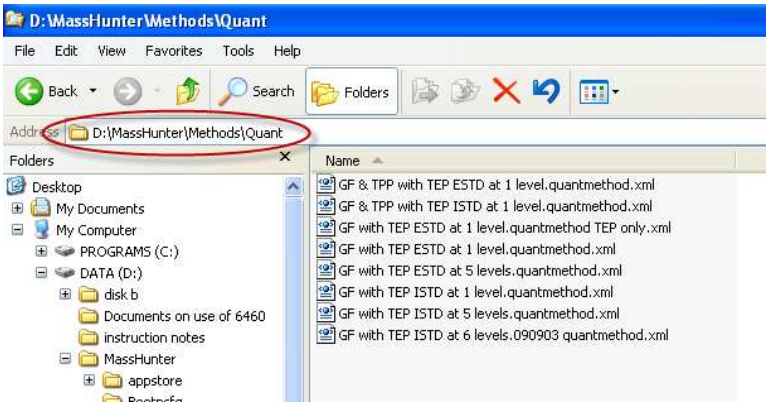

Figure 1.17c – quantitative analysis methods in D:\MassHunter\Methods\Quant folder

A column layout for Quantitative Analysis was created and stored. This layout shows certain columns (see figure 1.17e) in a multi-compound layout that is useful for reporting results from decon experiments. The most effective way to create a report from decon experiments is to choose this column layout, and then export the file to Excel where other calculations may then be performed.

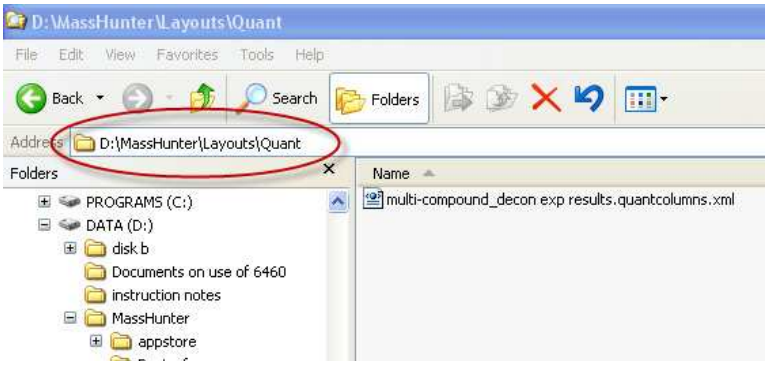

Figure 1.17d – quantitative analysis layout in D:\MassHunter\Layouts\Quant folder

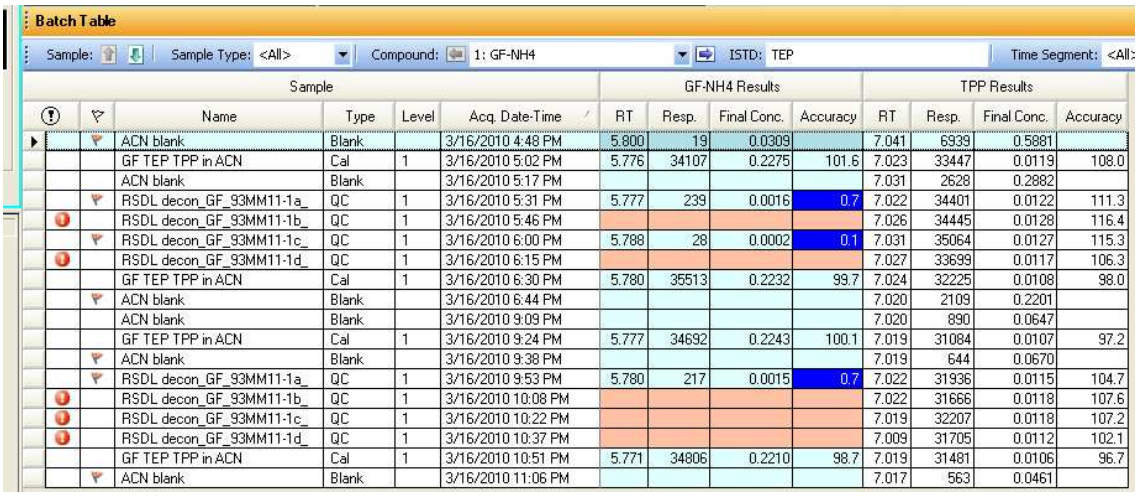

Figure 1.17e – quantitative analysis layout for multiple compounds, ready for export to Excel

Two work list templates were created, one for ESI and one for APCI. They are designed for analyzing the diluted solutions from a decon experiment, and they incorporate system flush methods at the end of the runs to clean out the system and prepare it for the next batch of sample analyses. Hardcopies of these worklists are presented in Annex 2. In order to use the worklists, the analyst should make changes to the Worklist Run Parameters to set the proper data path. Also, the Sample Positions and Data File names must be changed to reflect the current run. Setting the agent in solvent (100% standard) as Calibration sample type and the decon experiment solutions as QC sample type provides MassHunter Quantitative Analysis with the information to effectively process the batch. Details of processing a batch of samples are provided in a document, "Steps to processing samples - 6460 MassHunter.doc." A hardcopy of this document is provided in Annex 3.

Results from analysis of decon experiments can be calculated using ESTD or ISTD mode. It is recommended that ESTD be used, as this approach allows absolute tracking of recovery of surrogate compounds added at different stages of the decon experiment sample preparation and general ease of data interpretation.

1.18 Method write-up

A spreadsheet, "Decon Experiment Design - establish dilutions.xls," was developed to assist the analyst in determining the volumes required for performing the decon experiment and making appropriate dilutions for analysis on the 6460 LC-MS/MS system. A hardcopy of the printout is presented in Annex 4.

All methods for running the 6460 system are on the workstation and have been described in section 1.17.

Finally, the "Steps to processing samples - 6460 MassHunter.doc." document will assist the user in processing the batch of samples on the 6460 instrument.

These documents constitute the write-up of the method.

1.19 A draft report of the method validation suitable for submission to a peer reviewed journal

A draft manuscript of the analytical method suitable for publication was prepared. The recommended journal for publication is Journal of Chromatography A. A detailed guide for authors is located at:
http://www.elsevier.com/wps/find/journaldescription.cws_home/502688/authorinstructions

The draft manuscript can be fully populated with details from this final report. A hardcopy of the draft manuscript is provided in Annex 5.

Objective 2

2.1 Review screening procedures currently in place at DRDC Suffield

Meetings with the scientific authority were held to understand the process in place to conduct decon experiments.

2.2 Develop and write-up of a generic protocol, including chromatographic separation techniques for the components of interest; measurement and compensation techniques for any ion suppression/enhancement; selection of an appropriate internal standard; measurement of carryover, instrument precision and accuracy

The final LC-QQQ method developed has been shown to be applicable for both RSDL and F54 decon formulations. The recommended method is to use the Zorbax SB-C18 column (2.1x50mm, 1.8µ) and ESI. The Zorbax Eclipse XDB-C18 (4.6x50mm, 1.8µ) column can be used with ESI as a backup, or with APCI.

Generic protocols for use with decon formulations and CWAs were developed based on the findings of all aspects of this project.

The generic protocol for performing decon experiments with a decon formulations and CWAs not previously studied are presented in Annexes 6 and 7, respectively.

2.3 Testing of the generic protocol using one CW agent and one decontamination matrix on the 6460 QQQ

Testing of the decon experiment protocol and analytical method developed showed erratic results for TEP. Samples were processed using the Gilson automated liquid handler to minimize handling of potentially hazardous materials by the analyst. The results of this experiment, shown in table 2.3a, were not as expected. TEP results (area and recovery values) were variable, indicating problems with either the decon experiment design, the actual sample processing or instrumental analysis.

Sample	TEP		GF-NH4	
Name	Area	Recovery	Area	Recovery
AcCN Blank	250		442	
GF wTEP Std 1	222730		16779	
GF wTEP Std 2	228032		33422	
GF wTEP Std 3	243067		66583	
GF wTEP Std 4	259294		131342	
GF wTEP Std 5	229769		255098	
AcCN Blank	256		462	
British Decon 94MM191-1C	101541	54%	25	0%
British Decon 94MM191-1D	73227	39%	28	0%
British Decon 94MM191-1E	72159	38%		
British Decon 94MM191-1F	152162	81%		
British Decon 94MM191-1G	205320	109%	41	
GF wTEP Std 4	259000	0%	129634	0%
AcCN Blank	260	0%	416	0%
GF in AcCN – 100% 94MM193-1C	188269	100%	155942	100%
F54 in AcCN 94MM193-2C	105945	56%	132492	85%
F54 in tap H2O 94MM193-3C	139527	74%	132848	85%
Brit Decon mimic H2O 94MM195-1C	82255	44%	54075	35%
GF wTEP Std 4	257461	0%	127497	0%
GF wTEP Std 5	226131	0%	252309	0%
AcCN Blank	220	0%	457	0%
GF in AcCN – 100% 94MM193-1C	191323	102%	157993	101%

Table 2.3a – initial decon experiment showing variable TEP results

Various approaches were used to check the instrumental method to be sure that it was not the source of the problem. Analysis of further dilutions of the decon samples showed the same pattern of results, which indicated that ion suppression in the ESI source was not the cause of the problem (data not shown).

Problems were eventually traced to physical non-homogeneity within the decon sample vial. The solutions made with F54 resulted in two phases, and the Gilson unit was not providing adequate mixing during the experiment. Different mixing steps using the Gilson unit were investigated, however none worked as well or as consistently as shaking the vial by hand prior to withdrawing an aliquot. A wait step was added to the Gilson program to allow for this manual hand shaking step. The next decon experiment performed (see table 2.3b) showed more consistent results for TEP indicating the suitability of the sampling process as well as applicability of the instrumental detection method.

Sample	TEP		GF-NH4	
Name	Area	Recovery	Area	Recovery
AcCN blank	245			
GF in AcCN 7-1a	141402	97%	116768	97%
GF in Br Decon 5-1a	146700	100%		
GF in Br Decon 5-1b	113488	78%		
GF in Br Decon 5-1c	139868	96%		
GF in Br Decon 5-1d	141456	97%		
GF in Br Decon 5-1e	139310	95%		
GF in F54_water 7-2a	149947	102%	130987	108%
GF in F54_AcCN 7-3a	151115	103%	130245	108%
GF in Br Mimic 7-4a	133215	91%	85	0%

Table 2.3b – decon experiment with hand mixing of solutions

2.4 Refinements to the generic protocol

Mixing of the decon and diluted solutions is critical to success of the decon experiment. The Gilson automated liquid handler does not perform adequate mixing for all matrices.

A check on the physical solution dynamics was added to the generic protocol.

2.5 Adaption of the generic protocol for the 6130 MS single quad where possible

This step was not completed. See section 1.16 for explanation.

2.6 Testing of the generic protocol using one CW agent and one decontamination matrix on the 6130 single quad

This step was not completed as no work was performed on the 6130 single quad.

2.7 Any further refinements required to the generic protocol

None required.

2.8 Preparation of a generic work flow diagram/list to use with the protocol

A generic protocol for performing decon experiments with a decon formulation not previously studied is presented in Annex 6.

A generic protocol for performing decon experiments with a CWA not previously studied is presented in Annex 7.

2.9 Preparation of templates for work lists, methods and reports for Agilent MassHunter and ChemStation software

See section 1.10 for MassHunter templates. Instrumental methods developed are suitable for both RSDL and British Decon (containing F54) experiments.

2.10 Method write-up

Annexes 6 and 7 which contain the protocols for performing decon experiments constitute the write-up of the method.

2.11 A draft report of the method validation suitable for submission to a peer reviewed journal

The work performed for objective 2 is not suitable for publication in a peer reviewed journal at this time. Therefore no draft manuscript was prepared.

General Tasks Supporting Objectives 1 & 2

G1 Attend general EPG safety briefing.
Medical Countermeasures Briefing was attended on Dec. 7, 2009 and again on Jan. 14, 2010.

G2 Attend work specific safety briefing.
Work specific safety training was completed and the safety checklist signed on Dec. 7, 2009. See Annex 8 for the signed safety checklist.

G3 Observance of on-site safety, health and environmental standards on protection of property.
On-site health and safety measures were observed in performing this study, including use of personal protective equipment (glasses, gloves, lab coat) when handling vials that contain CW agents and proper disposal of labware that may contain CW agents.

G4 Complete and sign safety checklist.
See G2.

G5 Meet regularly with scientific authority.
Regular informal meetings were held with the scientific authority to provide updates on progress, discuss actions to be addressed and establish schedules.

G6 Monthly and final report preparation.
Monthly reports were prepared for Dec 2009, Jan and Feb 2010. Hardcopies of the report were submitted within one week following the last day of the month. Copies of these reports (hardcopy in Annex 9 and electronic) are provided with this final report.

G7 Other general activities supporting Objectives 1 & 2.

- LC maintenance was performed:
  - LC pressure fluctuations were observed. The autosampler needle seat and needle loop were backflushed, however, minor fluctuations were still evident. The needle seat was replaced, and the pressures remained consistent for the duration of the project.
  - LC tubing was changed from green (0.17 mm internal diameter) to red (0.12 mm internal diameter) in order to decrease system dead volume for improved chromatography with sub-2 micron particle columns.

- A high background noise issue was resolved by power cycling the MS.

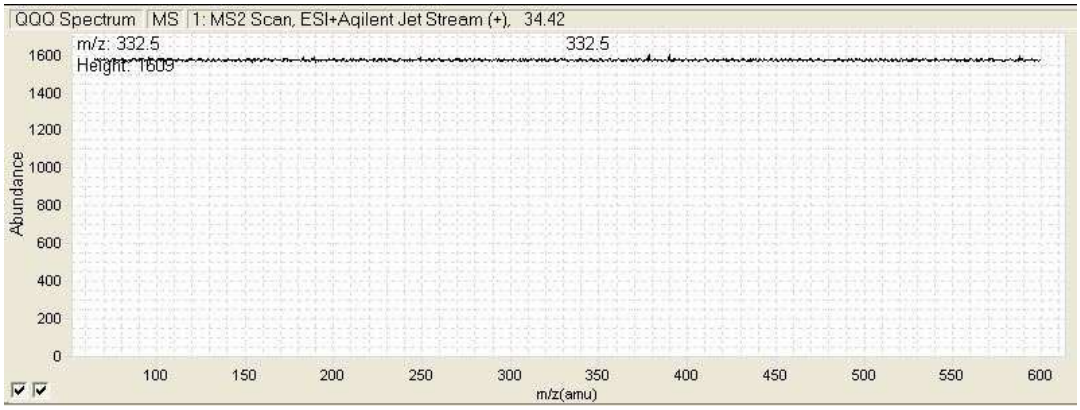

Figure G7a – high background noise

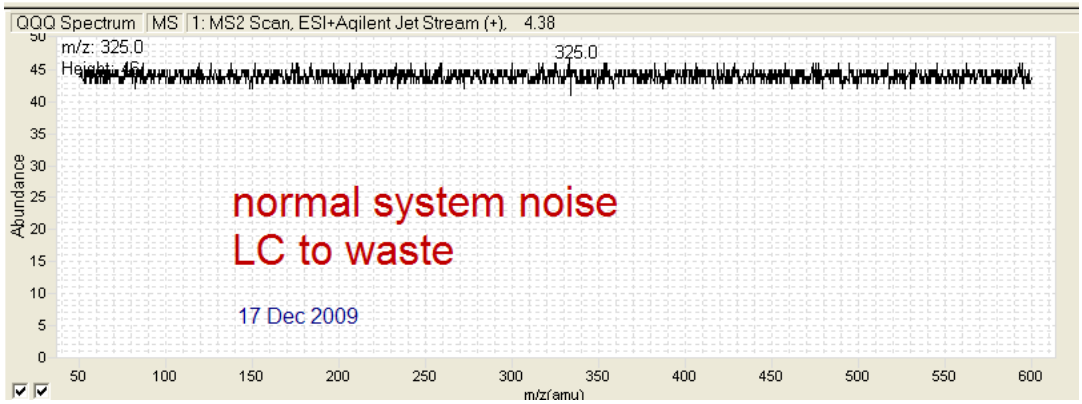

Figure G7b – normal background noise

- Column flush methods were prepared to ensure the system is properly cleaned after processing a batch of decon experiment samples. These methods contain somewhat odd gradient conditions. This is needed to ensure the organic solvent (either ACN or MeOH) is properly loaded in the pump head and therefore being sent to the column. The ACN column flush method should be used first, followed by the MeOH column flush method, to leave the pump and column ready for the next batch of analysis. Note that no MS data is collected during these runs.

- The ESI AJS nebulizer flush method was modified somewhat to be run between the ACN and MeOH column flushes. Run the method by injecting a blank (e.g. Vial 2 with DI H2O) to incorporate injector valve rotations for additional cleaning. Note that if no injection is made (using vial position -1), then the valve rotations do not occur. The full scan data collected during this nebulizer flush method can be used to compare system cleanliness over time.

- The recommended solution for the autosampler needle wash is a mixture of equal amounts of DI H2O, MeOH and IPA. This mixture can be adjusted with more or less water depending upon the solubility of agents and decon formulations.

- A system suitability check using TEP, TPP and TBP was established. The method involves injecting 1 µL of 0.01 ng/µL solution. The areas of each compound can be checked and tracked to monitor LC-QQQ performance. Use of a control chart (an example Excel template was loaded onto the LC-QQQ workstation) provides the ability to generate warning and control limits, and visually compare the results.

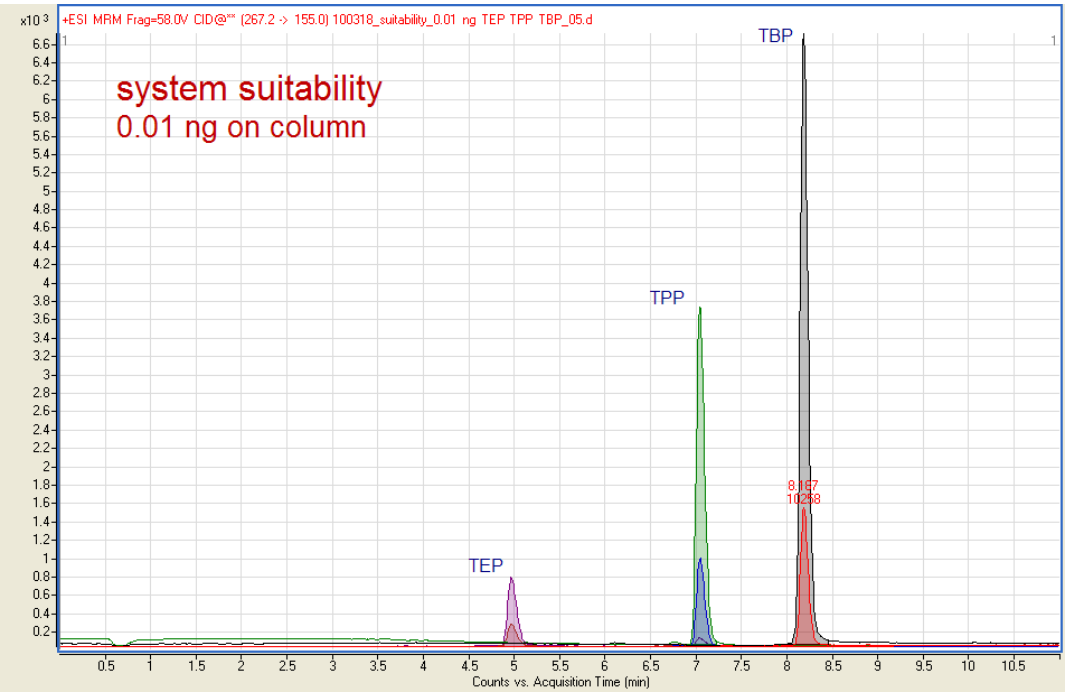

Figure G7c – example chromatography and response for system suitability check

List of Deliverables

Objectives 1 & 2

Monthly Progress Reports – provided in Annex 9.

Final report – provided in hardcopy and on CD-ROM

Draft manuscript of the method validation suitable for submission to a peer reviewed journal - provided in Annex 5

Software templates for work lists, LCMS method and reports for MassHunter – provided on CD-ROM and left on DRDC 6460 workstation

List of files on CD-ROM

Decon Experiment Design - establish dilutions.xls

draft Manuscript.doc

Final Report - contract W7702-09R230.doc

Final Report - contract W7702-09R230_Annexes.doc

generic protocol flowchart - CWA.docx

generic protocol flowchart - decon formulation.docx

Steps to processing samples - 6460 MassHunter.doc

MassHunter files:

Layouts\Quant

multi-compound_decon exp results.quantcolumns.xml

single-compound_decon exp results.quantcolumns.xml

Methods\!Decon Experiments

AJS-ESI_GD TEP TPP_MRM_SB-C18_100318.m

AJS-ESI_GF TEP TPP_MRM_SB-C18_100318.m

AJS-ESI_GF TEP TPP_MRM_XB-C18_100318.m

APCI_GF TEP TPP_MRM_XB-C18_100318.m

flush col 2.1 SB_ACN_ESI_100318.m

flush col 2.1 SB_MeOH_ESI_100318.m

flush col 4.6 XDB_ACN_APCI_100318.m

flush col 4.6 XDB_ACN_ESI_100318.m

flush col 4.6 XDB_MeOH_APCI_100318.m

flush col 4.6 XDB_MeOH_ESI_100318.m

flush ESI AJS source_ACN.m

system_suitability_3 phosphates_SB-C18.m

Worklists

!decon experiment example worklist_AJS-ESI.wkl

!decon experiment example worklist_APCI.wkl

Recommendations for Further Work

Breakdown product identification and develop methods for quantitative analysis.

Reduce matrix effects in ESI by investigating 2D LC techniques and / or sample clean-up procedures. This could render the single quad instrument useful for testing.

The Gilson automated liquid handler is a good tool for reducing the handling and exposure of toxic agents by DRDC staff. The system could be made more useful through custom programming to better handle decon solution testing.

Investigate the usefulness of other lab instruments for this work: 6130 single quad MS, ELSD.

Investigate the stability of agents in different solvents and storage conditions, filling gaps in literature.

Acknowledgements

The author would like to thank Michele Mayer and Dr. Paul D'Agostino of DRDC for sharing their knowledge regarding decontaminant solutions and analysis of chemical warfare agents, Ralph Hindle for sharing his knowledge on LC and MS/MS systems and Matthew Noestheden for assistance editing this report.

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   Robert W. Read, Robin M. Black
   Journal of Chromatography A, 862 (1999(, 169-177.

3. 20100010284About RSDL, RSDecon web page.
   http://www.rsdecon.com/pages/aboutUS.htm

4. Patent application title: DECONTAMINATION FORMULATIONS, web page.
   http://www.faqs.org/patents/app/

Annex 1 – MRM and system flush method printouts

AJS-ESI_GD TEP TPP_MRM_SB-C18_100318.m

AJS-ESI_GF TEP TPP_MRM_SB-C18_100318.m

AJS-ESI_GF TEP TPP_MRM_XB-C18_100318.m

APCI_GF TEP TPP_MRM_XB-C18_100318.m

flush col 2.1 SB_ACN_ESI_100318.m

flush col 2.1 SB_MeOH_ESI_100318.m

flush col 4.6 XDB_ACN_APCI_100318.m

flush col 4.6 XDB_ACN_ESI_100318.m

flush col 4.6 XDB_MeOH_APCI_100318.m

flush col 4.6 XDB_MeOH_ESI_100318.m

flush ESI AJS source_ACN.m

system_suitability_3 phosphates_SB-C18.m

Acquisition Method Report

Acquisition Method Info

Method Name AJS-ESI_GD TEP TPP_MRM_SB-C18_100318.m
Method Path D:\MassHunter\methods\!Decon Experiments\AJS-ESI_GD TEP TPP_MRM_SB-C18_100318.m
Method Description

MRM for NH4 adduct for GD, TEP & TPP, chromatography on SB-C18 2.1x50 mm 1.8u, 0.3 mL/min, 20%B 0-1 min, 20-100%B 1-8 min, flow 0.5 mL/min from 9-11 (stop), A=5 mM NH4Ac, B=5 mM NH4Ac in MeOH

Device List
h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source ESI+Agilent Jet Stream
Tune File atunes.tune.xml
Stop Mode No Limit/As Pump
Stop Time 1
Time Filter On
Time Filter Width 0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MRM	ESI+Agilent Jet Stream	To Waste	0	☐
2	3.5	MRM	ESI+Agilent Jet Stream	To MS	200	☑
3	8.5	MRM	ESI+Agilent Jet Stream	To Waste	0	☐

Time Segment 1

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Polarity
Compound1	☐	300	Unit	200	Unit	200	60	0	Positive

Fragmentor Ramp Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Time Segment 2

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Polarity
TPP	☑	225.1	Unit	141	Unit	90	58	4	Positive
TPP	☑	225.1	Unit	99	Unit	90	58	12	Positive
GD-NH4	☐	200.1	Unit	183.1	Unit	90	55	0	Positive
GD-NH4	☐	200.1	Unit	85	Unit	90	55	2	Positive
TEP	☐	183.1	Unit	127	Unit	90	70	6	Positive

TEP	☐	183.1	Unit	99	Unit	90	70	15	Positive

Fragmentor Ramp Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Time Segment **3**

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Polarity
Compound1	☐	300	Unit	200	Unit	200	60	0	Positive

Fragmentor Ramp Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	50000

Instrument Curves

Actual

Pump1 Current
Capillary
Gas Flow
Gas Temp
Sheath Gas Flow (l/min)
Sheath Gas Temp (°C)
Nebulizer

Wellplate Sampler

Name h-ALS-SL+ **Model** G1367D
Ordinal # 1 **Options** THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	0	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	3	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name BinPump-SL **Model** G1312B
Ordinal # 1 **Options** SSV

Stop Time (min) 11 **Post Time (min)** 4

Flow (ml/min) 0.3 **Pressure Min (bar)** 0

Pressure Max (bar) 400 **Max Flow Gradient (ml/min)** 100

Solvent A	5 mM NH4Ac	**Solvent B**	MeOH
Solvent Ratio A	80	**Solvent Ratio B**	20
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A	Auto	**Stroke B**	Auto

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
1	No Change	No Change	20
8	0.3	No Change	100
9	0.5	No Change	100

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Left Temp.	30	**Right Temp.**	Not Controlled
Left Ready	With Any Temp	**Right Ready**	0.8
Valve Position	1		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger
Contacts Time Table

Diode Array Detector

Name DAD-SL **Model** G1315C
Ordinal # 1 **Options**

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra	None	**Threshold**	10
Pre-Run Balance	No	**Post-Run Balance**	No
Balance Mode	1	**Margin for -ve absorbance**	100
Peak Width2	GT 0.1 min (2.0s)	**Wavelength**	4
Output Zero Offset1	5	**Output Zero Offset2**	5
Ouput Attenuation1	1000	**Output Attenuation2**	1000
UV Lamp	No	**Vis Lamp**	No
From	190	**To**	400
Step	2		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	200	4	0	0	Off
B	254	16	0	0	Off
C	210	8	0	0	Off
D	230	16	0	0	Off
E	280	16	0	0	Off
F	280	16	0	0	Off
G	280	16	0	0	Off
H	280	16	0	0	Off

Acquisition Method Report

Acquisition Method Info

Method Name AJS-ESI_GF TEP TPP_MRM_SB-C18_100318.m
Method Path D:\MassHunter\methods\!Decon Experiments\AJS-ESI_GF TEP TPP_MRM_SB-C18_100318.m
Method Description

MRM for NH4 adduct for GF, TEP & TPP, chromatography on SB-C18 2.1x50 mm 1.8u, 0.3 mL/min, 20%B 0-1 min, 20-100%B 1-8 min, flow 0.5 mL/min from 9-11 (stop), A=5 mM NH4Ac, B=5 mM NH4Ac in MeOH

Device List
h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source ESI+Agilent Jet Stream
Tune File atunes.tune.xml
Stop Mode No Limit/As Pump
Stop Time 1
Time Filter On
Time Filter Width 0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MRM	ESI+Agilent Jet Stream	To Waste	0	☐
2	3.5	MRM	ESI+Agilent Jet Stream	To MS	200	☑
3	8.5	MRM	ESI+Agilent Jet Stream	To Waste	0	☐

Time Segment **1**

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Polarity
Compound1	☐	300	Unit	200	Unit	200	60	0	Positive

Fragmentor Ramp Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Time Segment **2**

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Polarity
TPP	☑	225.1	Unit	141	Unit	90	58	4	Positive
TPP	☑	225.1	Unit	99	Unit	90	58	12	Positive
GF	☐	198.1	Unit	181.1	Unit	90	60	0	Positive
GF	☐	198.1	Unit	99	Unit	90	60	4	Positive
TEP	☐	183.1	Unit	127	Unit	90	70	6	Positive

TEP	☐	183.1	Unit	99	Unit	90	70	15	Positive

Fragmentor Ramp
Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Time Segment **3**

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Polarity
Compound1	☐	300	Unit	200	Unit	200	60	0	Positive

Fragmentor Ramp
Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	50000

Instrument Curves

Actual
Pump1 Current
Capillary
Gas Flow
Gas Temp
Sheath Gas Flow (l/min)
Sheath Gas Temp (°C)
Nebulizer

Wellplate Sampler

Name h-ALS-SL+ **Model** G1367D
Ordinal # 1 **Options** THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	0	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	3	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name BinPump-SL **Model** G1312B
Ordinal # 1 **Options** SSV

Stop Time (min) 11 **Post Time (min)** 4

Flow (ml/min) 0.3 **Pressure Min (bar)** 0

Pressure Max (bar) 400 **Max Flow Gradient (ml/min)** 100

Solvent A	5 mM NH4Ac	**Solvent B**	MeOH
Solvent Ratio A	80	**Solvent Ratio B**	20
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A	Auto	**Stroke B**	Auto

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
1	No Change	No Change	20
8	0.3	No Change	100
9	0.5	No Change	100

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Acquisition Method Report

Left Temp.	30	**Right Temp.**	Not Controlled
Left Ready	With Any Temp	**Right Ready**	0.8
Valve Position	1		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger
Contacts Time Table

Diode Array Detector

Name	DAD-SL	**Model**	G1315C
Ordinal #	1	**Options**	

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra	None	**Threshold**	10
Pre-Run Balance	No	**Post-Run Balance**	No
Balance Mode	1	**Margin for -ve absorbance**	100
Peak Width2	GT 0.1 min (2.0s)	**Wavelength**	4
Output Zero Offset1	5	**Output Zero Offset2**	5
Ouput Attenuation1	1000	**Output Attenuation2**	1000
UV Lamp	No	**Vis Lamp**	No
From	190	**To**	400
Step	2		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	200	4	0	0	Off
B	254	16	0	0	Off
C	210	8	0	0	Off
D	230	16	0	0	Off
E	280	16	0	0	Off
F	280	16	0	0	Off
G	280	16	0	0	Off
H	280	16	0	0	Off

Acquisition Method Report

Acquisition Method Info

Method Name AJS-ESI_GF TEP TPP_MRM_XB-C18_100318.m
Method Path D:\MassHunter\methods\!Decon Experiments\AJS-ESI_GF TEP TPP_MRM_XB-C18_100318.m
Method Description

MRM for NH4 adduct for GF, TEP & TPP, chromatography on XB-C18 4.6x50 mm 1.8u, 0.65mL/min, 20%B 0-1 min, 20-100%B 1-8 min, flow 0.8 mL/min from 9-11 (stop), A=5 mM NH4Ac, B=5 mM NH4Ac in MeOH

Device List
h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source ESI+Agilent Jet Stream
Tune File atunes.tune.xml
Stop Mode No Limit/As Pump
Stop Time 1
Time Filter On
Time Filter Width 0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MRM	ESI+Agilent Jet Stream	To Waste	0	☐
2	3.5	MRM	ESI+Agilent Jet Stream	To MS	200	☑
3	8.5	MRM	ESI+Agilent Jet Stream	To Waste	0	☐

Time Segment 1

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Polarity
Compound1	☐	300	Unit	200	Unit	200	60	0	Positive

Fragmentor Ramp Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Time Segment 2

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Polarity
TPP	☑	225.1	Unit	141	Unit	90	58	4	Positive
TPP	☑	225.1	Unit	99	Unit	90	58	12	Positive
GF	☐	198.1	Unit	181.1	Unit	90	60	0	Positive
GF	☐	198.1	Unit	99	Unit	90	60	4	Positive
TEP	☐	183.1	Unit	127	Unit	90	70	6	Positive

TEP	☐	183.1	Unit	99	Unit	90	70	15	Positive

Fragmentor Ramp
Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Time Segment **3**

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Polarity
Compound1	☐	300	Unit	200	Unit	200	60	0	Positive

Fragmentor Ramp
Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	50000

Instrument Curves

Actual

Pump1 Current
Capillary
Gas Flow
Gas Temp
Sheath Gas Flow (l/min)
Sheath Gas Temp (°C)
Nebulizer

Wellplate Sampler

Name h-ALS-SL+ **Model** G1367D
Ordinal # 1 **Options** THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	0	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	3	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name BinPump-SL **Model** G1312B
Ordinal # 1 **Options** SSV

Stop Time (min) 11 **Post Time (min)** 4

Flow (ml/min) 0.65 **Pressure Min (bar)** 0

Pressure Max (bar) 400 **Max Flow Gradient (ml/min)** 100

Solvent A 5 mM NH4Ac **Solvent B** MeOH
Solvent Ratio A 80 **Solvent Ratio B** 20

Compress. A (*10-6/bar) 100 **Compress. B (*10-6/bar)** 115
Stroke A Auto **Stroke B** Auto

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
1	No Change	No Change	20
8	0.65	No Change	100
9	0.8	No Change	100

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Left Temp.	30	**Right Temp.**	Not Controlled
Left Ready	With Any Temp	**Right Ready**	0.8
Valve Position	1		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger
Contacts Time Table

Diode Array Detector

Name	DAD-SL	**Model**	G1315C
Ordinal #	1	**Options**	

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra	None	**Threshold**	10
Pre-Run Balance	No	**Post-Run Balance**	No
Balance Mode	1	**Margin for -ve absorbance**	100
Peak Width2	GT 0.1 min (2.0s)	**Wavelength**	4
Output Zero Offset1	5	**Output Zero Offset2**	5
Ouput Attenuation1	1000	**Output Attenuation2**	1000
UV Lamp	No	**Vis Lamp**	No
From	190	**To**	400
Step	2		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	200	4	0	0	Off
B	254	16	0	0	Off
C	210	8	0	0	Off
D	230	16	0	0	Off
E	280	16	0	0	Off
F	280	16	0	0	Off
G	280	16	0	0	Off
H	280	16	0	0	Off

Acquisition Method Report

Acquisition Method Info

Method Name	APCI_GF TEP TPP_MRM_XB-C18_100318.m
Method Path	D:\MassHunter\methods\!Decon Experiments\APCI_GF TEP TPP_MRM_XB-C18_100318.m
Method Description	MRM for GF & TEP, NH4 adducts for GF, chromatography on XB-C18 4.6x50 mm 1.8u, 0.65 mL/min, 20%B initial, 20-100% from 1 to 8 min, A=5 mM NH4Ac, B=MeOH,

Device List

h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source	APCI
Tune File	atunes.tune.xml
Stop Mode	No Limit/As Pump
Stop Time	1
Time Filter	On
Time Filter Width	0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MRM	APCI	To MS	200	☑

Time Segment 1

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Polarity
GF-NH4	☐	198.1	Unit	181.1	Unit	90	60	0	Positive
GF-NH4	☐	198.1	Unit	99	Unit	90	60	5	Positive
TEP	☑	183.1	Unit	127	Unit	90	70	6	Positive
TEP	☑	183.1	Unit	99	Unit	90	70	15	Positive
GF	☐	181.1	Unit	99	Unit	90	60	0	Positive
GF	☐	181.1	Unit	81	Unit	90	60	32	Positive

Fragmentor Ramp

Source Parameters

Parameter	Value
Gas Temp (°C)	300
Vaporizer Temp (°C)	350
Gas Flow (l/min)	4
Nebulizer (psi)	40
Capillary (V)	3000
Corona Current Pos (µA)	10
Corona Current Neg (µA)	10

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	10000000

Instrument Curves

Actual

Pump1 Current
Capillary

Gas Flow

Actual
Gas Temp
Nebulizer

Wellplate Sampler

Name h-ALS-SL+ **Model** G1367D
Ordinal # 1 **Options** THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	0	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	3	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name BinPump-SL **Model** G1312B
Ordinal # 1 **Options** SSV

Stop Time (min) 11 **Post Time (min)** 4

Flow (ml/min) 0.65 **Pressure Min (bar)** 0

Pressure Max (bar) 400 **Max Flow Gradient (ml/min)** 100

Solvent A	5 mM NH4Ac in DI H2O	**Solvent B**	MeOH
Solvent Ratio A	80	**Solvent Ratio B**	20
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A	Auto	**Stroke B**	Auto

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B

1	No Change	No Change	20

Time	Flow	Pressure	Solv Ratio B
8	0.65	No Change	100
9	0.8	No Change	100

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Left Temp. 30 **Right Temp.** Not Controlled
Left Ready With Any Temp **Right Ready** 0.8
Valve Position 1

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger

Contacts Time Table

Diode Array Detector

Name DAD-SL **Model** G1315C
Ordinal # 1 **Options**

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra None
Pre-Run Balance No
Balance Mode 1
Peak Width2 GT 0.1 min (2.0s)
Output Zero Offset1 5
Ouput Attenuation1 1000
UV Lamp No
From 190
Step 2
Contact 1 0
Contact 2 0

Threshold 10
Post-Run Balance No
Margin for -ve absorbance 100
Wavelength 4
Output Zero Offset2 5
Output Attenuation2 1000
Vis Lamp No
To 400

Contact 3 0
Contact 4 0

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	200	4	0	0	Off
B	254	16	0	0	Off
C	210	8	0	0	Off
D	230	16	0	0	Off
E	280	16	0	0	Off
F	280	16	0	0	Off
G	280	16	0	0	Off
H	280	16	0	0	Off

Acquisition Method Report

Acquisition Method Info

Method Name	flush col 2.1 SB_ACN_ESI_100318.m
Method Path	D:\MassHunter\methods\!Decon Experiments\flush col 2.1 SB_ACN_ESI_100318.m
Method Description	flush column (2.1 x 50 mm, 1.8u) in position 1 with ACN - FOLLOW WITH MeOH COLUMN FLUSH METHOD!

Device List

h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source	ESI+Agilent Jet Stream
Tune File	atunes.tune.xml
Stop Mode	No Limit/As Pump
Stop Time	1
Time Filter	On
Time Filter Width	0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MS2 Scan	ESI+Agilent Jet Stream	To Waste	0	☐

Time Segment **1**

Scan Segments

Segment Name	Start Mass	End Mass	Scan Time	Frag (V)	Polarity
	50	400	400	60	Positive

Scan Parameters

Step Size	Data Stg	Threshold
0.1	Profile	0

Fragmentor Ramp

Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	10000000

Acquisition Method Report

Instrument Curves
Actual
Pump1 Current
Capillary
Gas Flow
Gas Temp
Nebulizer

Wellplate Sampler

Name h-ALS-SL+ **Model** G1367D
Ordinal # 1 **Options** THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	0	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	2	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name BinPump-SL **Model** G1312B
Ordinal # 1 **Options** SSV

Stop Time (min) 35 **Post Time (min)** Off

Flow (ml/min) 0.3 **Pressure Min (bar)** 0

Pressure Max (bar) 425 **Max Flow Gradient (ml/min)** 100

Solvent A	5 mM NH4Ac	**Solvent B**	ACN
Solvent Ratio A	80	**Solvent Ratio B**	20
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A	Auto	**Stroke B**	Auto

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
0.1	No Change	No Change	100
5	No Change	No Change	100
15	No Change	No Change	20
20	0.3	No Change	100
22	0.5	No Change	No Change
30	0.5	No Change	100
31	0.3	No Change	No Change
33	No Change	No Change	20

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Left Temp. 30 **Right Temp.** Not Controlled
Left Ready With Any Temp **Right Ready** 0.8
Valve Position 1

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger

Contacts Time Table

Diode Array Detector

Acquisition Method Report

Name DAD-SL **Model** G1315C
Ordinal # 1 **Options**

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra	None	**Threshold**	10
Pre-Run Balance	No	**Post-Run Balance**	No
Balance Mode	1	**Margin for -ve absorbance**	100
Peak Width2	GT 0.1 min (2.0s)	**Wavelength**	4
Output Zero Offset1	5	**Output Zero Offset2**	5
Ouput Attenuation1	1000	**Output Attenuation2**	1000
UV Lamp	No	**Vis Lamp**	No
From	190	**To**	400
Step	2		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	200	4	0	0	Off
B	254	16	0	0	Off
C	210	8	0	0	Off
D	230	16	0	0	Off
E	280	16	0	0	Off
F	280	16	0	0	Off
G	280	16	0	0	Off
H	280	16	0	0	Off

Acquisition Method Report

Acquisition Method Info

Method Name	flush col 2.1 SB_MeOH_ESI_100318.m
Method Path	D:\MassHunter\methods\!Decon Experiments\flush col 2.1 SB_MeOH_ESI_100318.m
Method Description	flush column in position 1 with MeOH

Device List
h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source	ESI+Agilent Jet Stream
Tune File	atunes.tune.xml
Stop Mode	No Limit/As Pump
Stop Time	1
Time Filter	On
Time Filter Width	0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MS2 Scan	ESI+Agilent Jet Stream	To Waste	0	☐

Time Segment 1

Scan Segments

Segment Name	Start Mass	End Mass	Scan Time	Frag (V)	Polarity
	50	400	400	60	Positive

Scan Parameters

Step Size	Data Stg	Threshold
0.1	Profile	0

Fragmentor Ramp

Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	10000000

Instrument Curves

Actual
Pump1 Current

Acquisition Method Report

Actual
Capillary
Gas Flow
Gas Temp
Nebulizer

Wellplate Sampler

Name h-ALS-SL+ **Model** G1367D
Ordinal # 1 **Options** THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	0	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	2	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name BinPump-SL **Model** G1312B
Ordinal # 1 **Options** SSV

Stop Time (min) 35 **Post Time (min)** Off

Flow (ml/min) 0.3 **Pressure Min (bar)** 0

Pressure Max (bar) 425 **Max Flow Gradient (ml/min)** 100

Solvent A	5 mM NH4Ac	**Solvent B**	MeOH
Solvent Ratio A	80	**Solvent Ratio B**	20
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A	Auto	**Stroke B**	Auto

Contact 1 0

Contact 2 0
Contact 3 0
Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
0.1	No Change	No Change	100
5	No Change	No Change	100
15	No Change	No Change	20
20	0.3	No Change	100
22	0.5	No Change	No Change
30	0.5	No Change	100
31	0.3	No Change	No Change
33	No Change	No Change	20

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Left Temp. 30 **Right Temp.** Not Controlled
Left Ready With Any Temp **Right Ready** 0.8
Valve Position 1

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger

Contacts Time Table

Diode Array Detector

Name DAD-SL **Model** G1315C

Acquisition Method Report

Ordinal # 1 **Options**

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra	None	**Threshold**	10
Pre-Run Balance	No	**Post-Run Balance**	No
Balance Mode	1	**Margin for -ve absorbance**	100
Peak Width2	GT 0.1 min (2.0s)	**Wavelength**	4
Output Zero Offset1	5	**Output Zero Offset2**	5
Ouput Attenuation1	1000	**Output Attenuation2**	1000
UV Lamp	No	**Vis Lamp**	No
From	190	**To**	400
Step	2		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	200	4	0	0	Off
B	254	16	0	0	Off
C	210	8	0	0	Off
D	230	16	0	0	Off
E	280	16	0	0	Off
F	280	16	0	0	Off
G	280	16	0	0	Off
H	280	16	0	0	Off

Acquisition Method Report

Acquisition Method Info

Method Name	flush col 4.6 XDB_ACN_APCI_100318.m
Method Path	D:\MassHunter\methods\!Decon Experiments\flush col 4.6 XDB_ACN_APCI_100318.m
Method Description	flush column (4.6 x 50mm 1.8 u) in position 1 with ACN - FOLLOW WITH MeOH COLUMN FLUSH METHOD!

Device List

h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source	APCI
Tune File	atunes.tune.xml
Stop Mode	No Limit/As Pump
Stop Time	1
Time Filter	On
Time Filter Width	0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MS2 Scan	APCI	To Waste	0	☐
Time Segment	**1**					

Scan Segments

Segment Name	Start Mass	End Mass	Scan Time	Frag (V)	Polarity
	50	400	400	60	Positive

Scan Parameters

Step Size	Data Stg	Threshold
0.1	Profile	0

Fragmentor Ramp

Source Parameters

Parameter	Value
Gas Temp (°C)	300
Vaporizer Temp (°C)	350
Gas Flow (l/min)	4
Nebulizer (psi)	40
Capillary (V)	3000
Corona Current Pos (µA)	10
Corona Current Neg (µA)	10

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	10000000

Acquisition Method Report

Instrument Curves
Actual
Pump1 Current
Capillary
Gas Flow
Gas Temp
Nebulizer

Wellplate Sampler

Name h-ALS-SL+ **Model** G1367D
Ordinal # 1 **Options** THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	0	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	2	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name BinPump-SL **Model** G1312B
Ordinal # 1 **Options** SSV

Stop Time (min) 35 **Post Time (min)** Off

Flow (ml/min) 0.5 **Pressure Min (bar)** 0

Pressure Max (bar) 450 **Max Flow Gradient (ml/min)** 100

Solvent A	5 mM NH4Ac	**Solvent B**	ACN
Solvent Ratio A	80	**Solvent Ratio B**	20
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A	Auto	**Stroke B**	Auto

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
0.1	No Change	No Change	100
5	No Change	No Change	100
15	No Change	No Change	20
20	0.5	No Change	100
22	0.8	No Change	No Change
30	0.8	No Change	100
31	0.5	No Change	No Change
33	No Change	No Change	20

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Left Temp. 30 **Right Temp.** Not Controlled
Left Ready With Any Temp **Right Ready** 0.8
Valve Position 1

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger

Contacts Time Table

Diode Array Detector

Acquisition Method Report

Name DAD-SL **Model** G1315C
Ordinal # 1 **Options**

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra	None	**Threshold**	10
Pre-Run Balance	No	**Post-Run Balance**	No
Balance Mode	1	**Margin for -ve absorbance**	100
Peak Width2	GT 0.1 min (2.0s)	**Wavelength**	4
Output Zero Offset1	5	**Output Zero Offset2**	5
Ouput Attenuation1	1000	**Output Attenuation2**	1000
UV Lamp	No	**Vis Lamp**	No
From	190	**To**	400
Step	2		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	200	4	0	0	Off
B	254	16	0	0	Off
C	210	8	0	0	Off
D	230	16	0	0	Off
E	280	16	0	0	Off
F	280	16	0	0	Off
G	280	16	0	0	Off
H	280	16	0	0	Off

Acquisition Method Report

Acquisition Method Info

Method Name	flush col 4.6 XDB_ACN_ESI_100318.m
Method Path	D:\MassHunter\methods\!Decon Experiments\flush col 4.6 XDB_ACN_ESI_100318.m
Method Description	flush column (4.6 x 50mm 1.8 u) in position 1 with ACN - FOLLOW WITH MeOH COLUMN FLUSH METHOD!

Device List
h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source	ESI+Agilent Jet Stream
Tune File	atunes.tune.xml
Stop Mode	No Limit/As Pump
Stop Time	1
Time Filter	On
Time Filter Width	0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MS2 Scan	ESI+Agilent Jet Stream	To Waste	0	☐

Time Segment 1

Scan Segments

Segment Name	Start Mass	End Mass	Scan Time	Frag (V)	Polarity
	50	400	400	60	Positive

Scan Parameters

Step Size	Data Stg	Threshold
0.1	Profile	0

Fragmentor Ramp

Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	3000
Charging Voltage (V)	500

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	10000000

Acquisition Method Report

Instrument Curves
Actual
Pump1 Current
Capillary
Gas Flow
Gas Temp
Nebulizer

Wellplate Sampler

Name h-ALS-SL+ **Model** G1367D
Ordinal # 1 **Options** THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	0	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	2	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name BinPump-SL **Model** G1312B
Ordinal # 1 **Options** SSV

Stop Time (min) 35 **Post Time (min)** Off

Flow (ml/min) 0.5 **Pressure Min (bar)** 0

Pressure Max (bar) 450 **Max Flow Gradient (ml/min)** 100

Solvent A	5 mM NH4Ac	**Solvent B**	ACN
Solvent Ratio A	80	**Solvent Ratio B**	20
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A	Auto	**Stroke B**	Auto

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
0.1	No Change	No Change	100
5	No Change	No Change	100
15	No Change	No Change	20
20	0.5	No Change	100
22	0.8	No Change	No Change
30	0.8	No Change	100
31	0.5	No Change	No Change
33	No Change	No Change	20

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Left Temp. 30 **Right Temp.** Not Controlled
Left Ready With Any Temp **Right Ready** 0.8
Valve Position 1

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger

Contacts Time Table

Diode Array Detector

Acquisition Method Report

Name DAD-SL **Model** G1315C
Ordinal # 1 **Options**

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra	None	**Threshold**	10
Pre-Run Balance	No	**Post-Run Balance**	No
Balance Mode	1	**Margin for -ve absorbance**	100
Peak Width2	GT 0.1 min (2.0s)	**Wavelength**	4
Output Zero Offset1	5	**Output Zero Offset2**	5
Ouput Attenuation1	1000	**Output Attenuation2**	1000
UV Lamp	No	**Vis Lamp**	No
From	190	**To**	400
Step	2		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	200	4	0	0	Off
B	254	16	0	0	Off
C	210	8	0	0	Off
D	230	16	0	0	Off
E	280	16	0	0	Off
F	280	16	0	0	Off
G	280	16	0	0	Off
H	280	16	0	0	Off

Acquisition Method Report

Acquisition Method Info

Method Name	flush col 4.6 XDB_MeOH_APCI_100318.m
Method Path	D:\MassHunter\methods\!Decon Experiments\flush col 4.6 XDB_MeOH_APCI_100318.m
Method Description	flush column (4.6 x 50mm 1.8 u) in position 1 with MeOH

Device List
h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source	APCI
Tune File	atunes.tune.xml
Stop Mode	No Limit/As Pump
Stop Time	1
Time Filter	On
Time Filter Width	0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MS2 Scan	APCI	To Waste	0	☐

Time Segment 1

Scan Segments

Segment Name	Start Mass	End Mass	Scan Time	Frag (V)	Polarity
	50	400	400	60	Positive

Scan Parameters

Step Size	Data Stg	Threshold
0.1	Profile	0

Fragmentor Ramp

Source Parameters

Parameter	Value
Gas Temp (°C)	300
Vaporizer Temp (°C)	350
Gas Flow (l/min)	4
Nebulizer (psi)	40
Capillary (V)	3000
Corona Current Pos (µA)	10
Corona Current Neg (µA)	10

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	10000000

Instrument Curves

Actual
Pump1 Current

Acquisition Method Report

Actual
Capillary
Gas Flow
Gas Temp
Nebulizer

Wellplate Sampler

Name h-ALS-SL+ **Model** G1367D
Ordinal # 1 **Options** THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	0	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	2	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name BinPump-SL **Model** G1312B
Ordinal # 1 **Options** SSV

Stop Time (min) 35 **Post Time (min)** Off

Flow (ml/min) 0.5 **Pressure Min (bar)** 0

Pressure Max (bar) 450 **Max Flow Gradient (ml/min)** 100

Solvent A	5 mM NH4Ac	**Solvent B**	MeOH
Solvent Ratio A	80	**Solvent Ratio B**	20
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A	Auto	**Stroke B**	Auto

Contact 1 0

Contact 2 0
Contact 3 0
Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
0.1	No Change	No Change	100
5	No Change	No Change	100
15	No Change	No Change	20
20	0.5	No Change	100
22	0.8	No Change	No Change
30	0.8	No Change	100
31	0.5	No Change	No Change
33	No Change	No Change	20

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Left Temp. 30 **Right Temp.** Not Controlled
Left Ready With Any Temp **Right Ready** 0.8
Valve Position 1

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger

Contacts Time Table

Diode Array Detector

Name DAD-SL **Model** G1315C

Acquisition Method Report

Ordinal # 1 **Options**

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra	None	**Threshold**	10
Pre-Run Balance	No	**Post-Run Balance**	No
Balance Mode	1	**Margin for -ve absorbance**	100
Peak Width2	GT 0.1 min (2.0s)	**Wavelength**	4
Output Zero Offset1	5	**Output Zero Offset2**	5
Ouput Attenuation1	1000	**Output Attenuation2**	1000
UV Lamp	No	**Vis Lamp**	No
From	190	**To**	400
Step	2		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	200	4	0	0	Off
B	254	16	0	0	Off
C	210	8	0	0	Off
D	230	16	0	0	Off
E	280	16	0	0	Off
F	280	16	0	0	Off
G	280	16	0	0	Off
H	280	16	0	0	Off

Acquisition Method Report

Acquisition Method Info

Method Name	flush col 4.6 XDB_MeOH_ESI_100318.m
Method Path	D:\MassHunter\methods\!Decon Experiments\flush col 4.6 XDB_MeOH_ESI_100318.m
Method Description	flush column (4.6 x 50mm 1.8 u) in position 1 with MeOH

Device List

h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source	ESI+Agilent Jet Stream
Tune File	atunes.tune.xml
Stop Mode	No Limit/As Pump
Stop Time	1
Time Filter	On
Time Filter Width	0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MS2 Scan	ESI+Agilent Jet Stream	To Waste	0	☐

Time Segment 1

Scan Segments

Segment Name	Start Mass	End Mass	Scan Time	Frag (V)	Polarity
	50	400	400	60	Positive

Scan Parameters

Step Size	Data Stg	Threshold
0.1	Profile	0

Fragmentor Ramp

Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	10000000

Instrument Curves

Actual

Pump1 Current

Acquisition Method Report

Actual
Capillary
Gas Flow
Gas Temp
Nebulizer

Wellplate Sampler

Name h-ALS-SL+ **Model** G1367D
Ordinal # 1 **Options** THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	0	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	2	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name BinPump-SL **Model** G1312B
Ordinal # 1 **Options** SSV

Stop Time (min) 35 **Post Time (min)** Off

Flow (ml/min) 0.5 **Pressure Min (bar)** 0

Pressure Max (bar) 450 **Max Flow Gradient (ml/min)** 100

Solvent A	5 mM NH4Ac	**Solvent B**	MeOH
Solvent Ratio A	80	**Solvent Ratio B**	20
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A	Auto	**Stroke B**	Auto

Contact 1 0

Contact 2 0
Contact 3 0
Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
0.1	No Change	No Change	100
5	No Change	No Change	100
15	No Change	No Change	20
20	0.5	No Change	100
22	0.8	No Change	No Change
30	0.8	No Change	100
31	0.5	No Change	No Change
33	No Change	No Change	20

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Left Temp. 30 **Right Temp.** Not Controlled
Left Ready With Any Temp **Right Ready** 0.8
Valve Position 1

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger

Contacts Time Table

Diode Array Detector

Name DAD-SL **Model** G1315C

Acquisition Method Report

Ordinal # 1 **Options**

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra	None	**Threshold**	10
Pre-Run Balance	No	**Post-Run Balance**	No
Balance Mode	1	**Margin for -ve absorbance**	100
Peak Width2	GT 0.1 min (2.0s)	**Wavelength**	4
Output Zero Offset1	5	**Output Zero Offset2**	5
Ouput Attenuation1	1000	**Output Attenuation2**	1000
UV Lamp	No	**Vis Lamp**	No
From	190	**To**	400
Step	2		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	200	4	0	0	Off
B	254	16	0	0	Off
C	210	8	0	0	Off
D	230	16	0	0	Off
E	280	16	0	0	Off
F	280	16	0	0	Off
G	280	16	0	0	Off
H	280	16	0	0	Off

Acquisition Method Report

Acquisition Method Info

Method Name flush ESI AJS source_ACN.m

Method Path D:\MassHunter\methods\!Decon Experiments\flush ESI AJS source_ACN.m

Method Description AcCN/water wash of nebulizer, no column, GF source conditions

Device List

h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source	ESI+Agilent Jet Stream
Tune File	atunes.tune.xml
Stop Mode	No Limit/As Pump
Stop Time	1
Time Filter	On
Time Filter Width	0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MS2 Scan	ESI+Agilent Jet Stream	To MS	0	☑

Time Segment 1

Scan Segments

Segment Name	Start Mass	End Mass	Scan Time	Frag (V)	Polarity
	50	1000	500	60	Positive

Scan Parameters

Step Size	Data Stg	Threshold
0.1	Profile	0

Fragmentor Ramp

Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	5
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	10000000

Acquisition Method Report

Instrument Curves
Actual
Pump1 Current

Wellplate Sampler

Name	h-ALS-SL+	**Model**	G1367D
Ordinal #	1	**Options**	THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	1	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	2	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name	BinPump-SL	**Model**	G1312B
Ordinal #	1	**Options**	SSV

Stop Time (min) 5 **Post Time (min)** Off

Flow (ml/min) 0.3 **Pressure Min (bar)** 0

Pressure Max (bar) 400 **Max Flow Gradient (ml/min)** 100

Solvent A	5 mM NH4Ac	**Solvent B**	ACN
Solvent Ratio A	10	**Solvent Ratio B**	90
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A	Auto	**Stroke B**	Auto

Contact 1 0
Contact 2 0
Contact 3 0

Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
0	0.3	No Change	No Change
0.5	2	No Change	No Change

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Left Temp. 30 **Right Temp.** Not Controlled
Left Ready With Any Temp **Right Ready** 0.8
Valve Position 0

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger

Contacts Time Table

Diode Array Detector

Name DAD-SL **Model** G1315C
Ordinal # 1 **Options**

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra None **Threshold** 10
Pre-Run Balance No **Post-Run Balance** No
Balance Mode 1 **Margin for -ve absorbance** 100

Acquisition Method Report

Peak Width2	GT 0.1 min (2.0s)	**Wavelength**	4
Output Zero Offset1	5	**Output Zero Offset2**	5
Ouput Attenuation1	1000	**Output Attenuation2**	1000
UV Lamp	No	**Vis Lamp**	No
From	190	**To**	400
Step	2		
Contact 1 0			
Contact 2 0			
Contact 3 0			
Contact 4 0			

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	**250**	**4**	**0**	**0**	**Off**
B	**254**	**16**	**0**	**0**	**Off**
C	**210**	**8**	**0**	**0**	**Off**
D	**230**	**16**	**0**	**0**	**Off**
E	**280**	**16**	**0**	**0**	**Off**
F	**280**	**16**	**0**	**0**	**Off**
G	**280**	**16**	**0**	**0**	**Off**
H	**280**	**16**	**0**	**0**	**Off**

Acquisition Method Report

Acquisition Method Info

Method Name	system_suitability_3 phosphates_SB-C18.m
Method Path	D:\MassHunter\methods\!Decon Experiments\system_suitability_3 phosphates_SB-C18.m
Method Description	MRM for TEP, TPP & TBP, chromatography on SB-C18 2.1x50 mm 1.8u, 0.3 mL/min, 20%B 0-1 min, 20-100%B 1-8 min, stop 11 min, A=5 mM NH4Ac, B=5 mM NH4Ac in MeOH

Device List

h-ALS-SL+
BinPump-SL
Column-SL
DAD-SL
MS QQQ

QQQ Mass Spectrometer

Ion Source	ESI+Agilent Jet Stream
Tune File	atunes.tune.xml
Stop Mode	No Limit/As Pump
Stop Time	1
Time Filter	On
Time Filter Width	0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MRM	ESI+Agilent Jet Stream	To MS	200	☑

Time Segment 1

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Polarity
TBP	☐	267.2	Unit	155	Unit	90	58	4	Positive
TBP	☐	267.2	Unit	99	Unit	90	58	12	Positive
TPP	☐	225.1	Unit	141	Unit	90	58	4	Positive
TPP	☐	225.1	Unit	99	Unit	90	58	12	Positive
TEP	☐	183.1	Unit	127	Unit	90	70	6	Positive
TEP	☐	183.1	Unit	99	Unit	90	70	15	Positive

Fragmentor Ramp

Source Parameters

Parameter	Value
Gas Temp (°C)	300
Gas Flow (l/min)	4
Nebulizer (psi)	50
Sheath Gas Temp (°C)	250
Sheath Gas Flow (l/min)	10
Capillary (V)	2500
Charging Voltage (V)	500

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	50000

Instrument Curves

Actual

Pump1 Current
Capillary

Gas Flow

Actual
Gas Temp
Sheath Gas Flow (l/min)
Sheath Gas Temp (°C)
Nebulizer

Wellplate Sampler

Name h-ALS-SL+ **Model** G1367D
Ordinal # 1 **Options** THM

Stop Time (min) As Pump **Post Time (min)** Off

Injection Type	Needle Wash	**Injection Volume**	1
Overlap Time	Disable Overlapped Injection	**Draw Position**	
Draw Position Detection	0	**Draw Speed**	
Eject Speed		**Flush Out Factor**	5
Enable Bypass	No	**Wait After Draw**	0
Wash Vessel	N/A	**Wash Location**	FlushPort
Wash Time	2	**Wash Cycles**	N/A
Ready Temp. Range		**Temp.**	

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Injector Program
Signals Selected
Contacts Time Table

Binary Pump

Name BinPump-SL **Model** G1312B
Ordinal # 1 **Options** SSV

Stop Time (min) 11 **Post Time (min)** 4

Flow (ml/min) 0.3 **Pressure Min (bar)** 0

Pressure Max (bar) 400 **Max Flow Gradient (ml/min)** 100

Solvent A	5 mM NH4Ac	**Solvent B**	MeOH
Solvent Ratio A	80	**Solvent Ratio B**	20
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A	Auto	**Stroke B**	Auto

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
1	No Change	No Change	20
8	No Change	No Change	100

Signals Selected

Description
Pressure
Flow
Solvent% B

Contacts Time Table

Thermostated Column Compartment

Name Column-SL **Model** G1316B
Ordinal # 1 **Options** CSV

Stop Time (min) As Pump **Post Time (min)** Off

Left Temp. 30 **Right Temp.** Not Controlled
Left Ready With Any Temp **Right Ready** 0.8
Valve Position 1

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Temperature Time Table
Signals Selected

Description
Temperature of left heat exchanger

Contacts Time Table

Diode Array Detector

Name DAD-SL **Model** G1315C
Ordinal # 1 **Options**

Stop Time (min) As Pump **Post Time (min)** Off **Delay Time (min)** 0

Store Spectra	None	**Threshold**	10
Pre-Run Balance	No	**Post-Run Balance**	No
Balance Mode	1	**Margin for -ve absorbance**	100
Peak Width2	GT 0.1 min (2.0s)	**Wavelength**	4
Output Zero Offset1	5	**Output Zero Offset2**	5
Ouput Attenuation1	1000	**Output Attenuation2**	1000
UV Lamp	No	**Vis Lamp**	No
From	190	**To**	400
Step	2		

Contact 1 0
Contact 2 0
Contact 3 0
Contact 4 0

Signal Time Table
Signals Selected
Contacts Time Table

Wavelength Settings

Channel	Sample WL	Sample BW	Ref. WL	Ref. BW	Ref. On
A	200	4	0	0	Off
B	254	16	0	0	Off
C	210	8	0	0	Off
D	230	16	0	0	Off
E	280	16	0	0	Off
F	280	16	0	0	Off
G	280	16	0	0	Off
H	280	16	0	0	Off

Annex 2 – MassHunter Work List templates

Worklist Report

Worklist Path:	**D:\MassHunter\Worklists\!decon experiment example worklist_AJS-ESI.wkl**

Worklist Run Parameters

Operator Name:	---
Run Type:	**Standard Start**
Part of Method to Run:	**Acquisition Only**
Execution for Acquisition-DA:	**Synchronous**
Acquisition Method Path:	**D:\MassHunter\methods\!Decon Experiments**
DA Method Path:	**D:\MassHunter\methods**
Data File Path:	**D:\MassHunter\data\2010**
Pre-Worklist Script:	---
Post-Worklist Script:	---
Acquisition Clean Up Script:	**SCP_InstrumentStandby(){MH_Acq_Scripts.exe}**
Overlapped Injection:	**Yes**
Clear sample selection after run:	**Yes**
Wait Time for Ready(Min):	**10**
Threshold Disk Value(GB):	**10**
Comment:	---

Worklist Table

Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Comment
ACN blank	P1-A1	GF TEP TPP_ESI_MRM_SB-C18_100318.m	ACN blank_01.d	Blank		
GF TEP TPP Std in ACN	P1-A2	GF TEP TPP_ESI_MRM_SB-C18_100318.m	GF TEP TPP Std in ACN_02.d	Calibration	1	
ACN blank	P1-A1	GF TEP TPP_ESI_MRM_SB-C18_100318.m	ACN blank_03.d	Blank		
decon solution 1_	P1-B1	GF TEP TPP_ESI_MRM_SB-C18_100318.m	decon solution 1_04.d	QC	1	
decon solution 2_	P1-B2	GF TEP TPP_ESI_MRM_SB-C18_100318.m	decon solution 2_05.d	QC	1	
decon solution 3_	P1-B3	GF TEP TPP_ESI_MRM_SB-C18_100318.m	decon solution 3_06.d	QC	1	
decon solution	P1-B4	GF TEP TPP_ESI_MRM_SB-	decon solution	QC	1	

4_		C18_100318.m	4_07.d			
GF TEP TPP Std in ACN	P1-A2	GF TEP TPP_ESI_MRM_SB-C18_100318.m	GF TEP TPP Std in ACN08.d	Calibration	1	
decon solution 5_	P1-C1	GF TEP TPP_ESI_MRM_SB-C18_100318.m	decon solution 5_09.d	QC	1	
decon solution 6_	P1-C2	GF TEP TPP_ESI_MRM_SB-C18_100318.m	decon solution 6_10.d	QC	1	
decon solution 7_	P1-C3	GF TEP TPP_ESI_MRM_SB-C18_100318.m	decon solution 7_11.d	QC	1	
decon solution 8_	P1-C4	GF TEP TPP_ESI_MRM_SB-C18_100318.m	decon solution 8_12.d	QC	1	
GF TEP TPP Std in ACN	P1-A2	GF TEP TPP_ESI_MRM_SB-C18_100318.m	GF TEP TPP Std in ACN13.d	Calibration	1	
ACN blank	P1-A1	GF TEP TPP_ESI_MRM_SB-C18_100318.m	ACN blank14.d	Blank		
column flush ACN	-1	flush col 2.1 SB_ACN_ESI_100318.m	column flush ACN.d	Sample		
ESI Agilent Jet Spray flush ACN	Vial 2	flush ESI AJS source_ACN.m	nebulizer flush ACN.d	Sample		
column flush MeOH	-1	flush col 2.1 SB_MeOH_ESI_100318.m	column flush MeOH.d	Sample		

Worklist Report

Worklist Path: **D:\MassHunter\Worklists\!decon experiment example worklist_APCI.wkl**

Worklist Run Parameters

Operator Name:	---
Run Type:	**Standard Start**
Part of Method to Run:	**Acquisition Only**
Execution for Acquisition-DA:	**Synchronous**
Acquisition Method Path:	**D:\MassHunter\methods\!Decon Experiments**
DA Method Path:	**D:\MassHunter\methods**
Data File Path:	**D:\MassHunter\data\2010**
Pre-Worklist Script:	---
Post-Worklist Script:	---
Acquisition Clean Up Script:	**SCP_InstrumentStandby(){MH_Acq_Scripts.exe}**
Overlapped Injection:	**Yes**
Clear sample selection after run:	**Yes**
Wait Time for Ready(Min):	**10**
Threshold Disk Value(GB):	**10**
Comment:	---

Worklist Table

Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Comment
ACN blank	P1-A1	GF TEP TPP_APCI_MRM_XB-C18_100318.m	ACN blank_01.d	Blank		
GF TEP TPP Std in ACN	P1-A2	GF TEP TPP_APCI_MRM_XB-C18_100318.m	GF TEP TPP Std in ACN_02.d	Calibration	1	
ACN blank	P1-A1	GF TEP TPP_APCI_MRM_XB-C18_100318.m	ACN blank_03.d	Blank		
decon solution 1_	P1-B1	GF TEP TPP_APCI_MRM_XB-C18_100318.m	decon solution 1_04.d	QC	1	
decon solution 2_	P1-B2	GF TEP TPP_APCI_MRM_XB-C18_100318.m	decon solution 2_05.d	QC	1	
decon solution 3_	P1-B3	GF TEP TPP_APCI_MRM_XB-C18_100318.m	decon solution 3_06.d	QC	1	
decon solution 4_	P1-B4	GF TEP TPP_APCI_MRM_XB-C18_100318.m	decon solution 4_07.d	QC	1	
GF TEP TPP Std	P1-A2	GF TEP TPP_APCI_MRM_XB-C18_100318.m	GF TEP TPP Std in	Calibration	1	

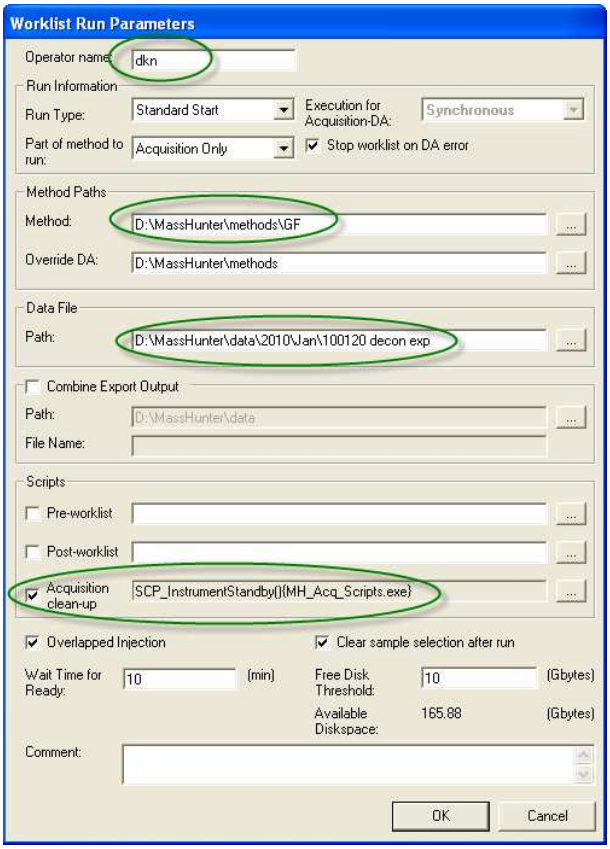
in ACN			ACN08.d			
decon solution 5_	P1-C1	GF TEP TPP_APCI_MRM_XB-C18_100318.m	decon solution 5_09.d	QC	1	
decon solution 6_	P1-C2	GF TEP TPP_APCI_MRM_XB-C18_100318.m	decon solution 6_10.d	QC	1	
decon solution 7_	P1-C3	GF TEP TPP_APCI_MRM_XB-C18_100318.m	decon solution 7_11.d	QC	1	
decon solution 8_	P1-C4	GF TEP TPP_APCI_MRM_XB-C18_100318.m	decon solution 8_12.d	QC	1	
GF TEP TPP Std in ACN	P1-A2	GF TEP TPP_APCI_MRM_XB-C18_100318.m	GF TEP TPP Std in ACN13.d	Calibration	1	
ACN blank	P1-A1	GF TEP TPP_APCI_MRM_XB-C18_100318.m	ACN blank14.d	Blank		
column flush ACN	-1	flush col 4.6 XDB_ACN_APCI_100318.m	column flush ACN.d	Sample		
column flush MeOH	-1	flush col 4.6 XDB_MeOH_APCI_100318.m	column flush MeOH.d	Sample		

Annex 3 – Steps to processing samples - 6460 MassHunter.doc

Steps to processing samples:

In Worklist:

- Create a New Worklist
- Set Worklist Run Parameters:
  - Operator
  - Method folder
  - Data folder
  - Acquisition clean-up script
- Enter sample information, setting the 100% agent vial as a Calibration sample type and level 1, and decon solutions as QC sample type, level 1
- Run the samples on the 6460



		Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Comment
1	✔	AcCN Blank - fresh	P1-A9	GF_ESI_MRM_100118.m	AcCN Blank_01.d	Blank		
2	✔	GF in AcCN - 100% 94MM193-1C	P1-C1	GF_ESI_MRM_100118.m	GF in AcCN - 100_94MM193-1C_02.d	Calibration	1	
3	✔	F54 in AcCN 94MM193-2C	P1-C2	GF_ESI_MRM_100118.m	F54 in AcCN_94MM193-2C_03.d	QC	1	
4	✔	F54 in tap H2O 94MM193-3C	P1-C3	GF_ESI_MRM_100118.m	F54 in tap H2O_94MM193-3C_04.d	QC	1	
5	✔	Brit Decon mimic H2O 94MM195-1C	P1-C4	GF_ESI_MRM_100118.m	Brit Decon mimic_94MM195-1C_05.d	QC	1	
6	✔	AcCN Blank - fresh	P1-A9	GF_ESI_MRM_100118.m	AcCN Blank_06.d	Blank		
7	✔	GF in AcCN - 100% 94MM193-1C	P1-C1	GF_ESI_MRM_100118.m	GF in AcCN - 100_94MM193-1C_07.d	QC	1	

In Quant:

- Create a New Batch – navigate to the data folder and create a descriptive name for the batch in the same folder
- Add Samples to the batch – choose Select All or select files using shift & control keys
- Apply a Quant method by choosing Method | Open
  - Choose a method from an existing file in the MassHunter\Methods\Quant folder or from an existing batch (be careful as any specific changes made to the method for that batch will be used for this new one).
- From the Method Edit view, review the method if you wish, and then click Exit, and Yes to apply the method to the batch.
- In the batch at a glance view, click the Analyse Batch button.
- Review the data and make any necessary changes (curve fit, etc.).
- Save the batch.
- Use File | Export | Export Table… to send the quant batch results to an Excel file.

Annex 4 – Decon Experiment Design - establish dilutions.xls

Decon Experiment design

Date: _____

Initials: _____

Prepare starting solutions:

Solution	volume (uL)	of Stock (ng/uL)	Volume solvent (uL)	solvent	Concentration (ng/uL)
F-54	1000	100%	9000	ACN	10%
GF	100	12000	900	ACN	1064.65
TEP	100	24000	900	ACN	1847

Decon experiment

Solution	volume (uL)	of Stock (ng/uL)	Volume solvent (uL)	solvent	Concentration (ng/uL)
F-54	600	10%	0	ACN	6%
GF	350	1064.65	0	ACN	372.63
TEP	50	1847	0	ACN	92.35

total: *1000*

Intermediate dilution

Components	volume (uL)	of Stock (ng/uL)	Volume solvent (uL)	solvent	Concentration (ng/uL)
volumes -->	50		950	ACN	
F-54		6.00%			0.30%
GF		372.6275			18.63
TEP		92.35			4.62

total: *1000*

Dilution for analysis on 6460

Solution	volume (uL)	of Stock (ng/uL)	Volume solvent (uL)	solvent	Concentration (ng/uL)
volumes -->	50		950	ACN	
F-54		0.30%			0.015%
GF		18.631375			0.932
TEP		4.6175			0.231

total: *1000*

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Decon Experiment - Standards

Initial Stock Dilutions

Solution	volume (uL)	of Stock (ng/uL)	Concentration (ng/uL)
solvent - ACN	825		
GF	175	1064.65	186.31
total:	*1000*		

Solution	volume (uL)	of Stock (ng/uL)	Concentration (ng/uL)
solvent - ACN	950		
TEP	50	1847	92.35
total:	*1000*		

Intermediate Stock Dilutions

Solution	volume (uL)	of Stock (ng/uL)	Concentration (ng/uL)
solvent - ACN	975		
GF	25	186.31375	4.66
total:	*1000*		

Solution	volume (uL)	of Stock (ng/uL)	Concentration (ng/uL)
solvent - ACN	975		
TEP	25	92.35	2.31
total:	*1000*		

Calibration Standard 1

Solution	volume (uL)	of Stock (ng/uL)	Concentration (ng/uL)
solvent - ACN	700		
GF	200	4.66	0.932
TEP	100	2.31	0.231
total:	*1000*		

Calibration Standard 2

Solution	volume (uL)	of Stock (ng/uL)	Concentration (ng/uL)
solvent - ACN	800		
GF	100	4.66	0.466
TEP	100	2.31	0.231
total:	*1000*		

Calibration Standard 3

Solution	volume (uL)	of Stock (ng/uL)	Concentration (ng/uL)
solvent - ACN	850		
GF	50	4.66	0.233
TEP	100	2.31	0.231
total:	*1000*		

Calibration Standard 4

Solution	volume (uL)	of Stock (ng/uL)	Concentration (ng/uL)
solvent - ACN	875		
GF	25	4.66	0.116
TEP	100	2.31	0.231
total:	*1000*		

Calibration Standard 5

Solution	volume (uL)	of Stock (ng/uL)	Concentration (ng/uL)
solvent - ACN	887.5		
GF	12.5	4.66	0.0582
TEP	100	2.31	0.231
total:	*1000*		

Decon Experiment design - neat

Starting solutions:

Solution	Concentration (% or ng/uL)
F-54 in British Decon	20%
GF	100%
TEP	18345

Decon experiment

Solution	volume (uL)	of Stock (ng/uL)	Volume solvent (uL)	solvent	Concentration (% or ng/uL)
F-54 in British Decon	970	20%	0		19.4%
GF	30	100%	0		3.0%
TEP	0	18345	0		0.00
total:	1000				

Initial dilution

Components	volume (uL)	of Stock (ng/uL)	Volume solvent (uL)	solvent	Concentration (%)
volumes -->	25		965	ACN	
F-54 in British Decon		19.40%			0.49%
GF		3.0%			0.075%
TEP	10	18345			183.5
total:	*1000*				

Intermediate dilution

Solution	volume (uL)	of Stock (ng/uL)	Volume solvent (uL)	solvent	Concentration (% or ng/uL)
volumes -->	25		975	ACN	
F-54 in British Decon		0.49%			0.01213%
GF		0.075%			0.00188%
TEP		183.5			4.59
total:	*1000*				

Dilution for analysis on 6460

Solution	volume (uL)	of Stock (ng/uL)	Volume solvent (uL)	solvent	Concentration (% or ng/uL)
volumes -->	50		950	ACN	
F-54 in British Decon		0.01213%			0.00061%
GF		0.00188%			0.938
TEP		4.59			0.229
total:	*1000*				

Annex 5 – Draft manuscript suitable for publication in a peer reviewed journal

Journal of Chromatography A – guide for Authors

As part of the Introduction section to each manuscript, authors must address the question of how their proposed methodology compares with previously reported methods and this comparison must show that significant advances are proposed.

Analytical performance characteristics of new methods should be given, including sensitivity, tested limits of detection or quantification, accuracy, precision, and specificity

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to "the text". Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on.

Title. Concise and informative. Titles are often used in information-retrieval systems. Do not include abbreviations or trade names in the title.

Author names and affiliations. Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name, and, if available, the e-mail address of each author.

Corresponding author. Clearly indicate who is willing to handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address.**

Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a "Present address"' (or "Permanent address") may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and", "of"). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Nomenclature and units

Widely accepted symbols, abbreviations and units (SI) should be used. Symbols and abbreviations that need not be defined are listed in Appendix 1. For all other symbols and abbreviations, the full expression followed by the abbreviation should be given the first time it appears in the text. Abbreviations used in tables and figures should be explained in the captions. In general, the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) should be followed (see http://www.iupac.org) and attention should be given to the recommendations of the Analytical Chemistry Division in the journal Pure and Applied Chemistry: Nomenclature for Chromatography, Pure Appl. Chem.,65 (1993) 819-872. Special attention should be given to the use of the terms **repeatability** and **reproducibility**; these are often confused.

Decimal points should be indicated by full stops. All decimal numbers smaller than unity should include a leading zero (e.g. 0.11). Company-specific research codes for compounds should not be used; after a full definition of the compound (possibly including such codes) in the Introduction, it may be further indicated by a bold-face Roman or Arabic numeral.
Appendix 1: Abbreviations and symbols that may be used without definition

Math formulae

Present simple formulae in the line of normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

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Table footnotes
Indicate each footnote in a table with a superscript lowercase letter.

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Electronic artwork

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• Number the illustrations according to their sequence in the text.
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Simple straight-line graphs (such as calibration lines) are not acceptable, because they can readily be described in the text by means of an equation or a sentence. Claims of linearity should be supported by regression data that include slope, intercept, standard deviations of the slope and intercept, standard error and the number of data points; correlation coefficients are optional. Standard symbols should be used in line drawings; the following are available to the typesetters and can also be used in the legends: filled or open squares, triangles, circles or diamonds, + or x.

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Tables

Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

References

Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either "Unpublished results" or "Personal communication" Citation of a reference as "in press" implies that the item has been accepted for publication.

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Reference Style

Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.
Example: "..... as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result ...."
List: Number the references (numbers in square brackets) in the list in the order in which they appear in the text.
Examples:
Reference to a journal publication:
[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, J. Sci. Commun. 163 (2000) 51.
Reference to a book:
[2] W. Strunk Jr., E.B. White, The Elements of Style, Macmillan, New York, 3rd ed., 1979.
Reference to a chapter in an edited book:
[3] G.R. Mettam, L.B. Adams, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing, New York, 1994, pp. 281.

Journal abbreviations source
Journal names should be abbreviated according to
Index Medicus journal abbreviations: http://www.nlm.nih.gov/tsd/serials/lji.html;
List of serial title word abbreviations: http://www.issn.org/2-22661-LTWA-online.php;
CAS (Chemical Abstracts Service): http://www.cas.org/sent.html.

Determination of the chemical warfare agent GF in decontamination formulations by liquid chromatography-tandem mass spectrometry.

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Abstract

Decontamination (decon) formulations for chemical warfare agents must be tested under various conditions to prove efficacy. Testing for residues of the agent in the presence of the decon solution can be challenging, especially in the short time frames in which deactivation of the agent should occur. A method was developed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electrospray ionization for the analysis of GF. The method was tested using two different decontaminant formulations. The method was shown to be free from matrix effects with appropriate dilutions of the decon solutions. Tripropyl phosphate and Triethyl phosphate were used to monitor effectiveness of the decon experiment sample process and LC-MS/MS method. The limit of detection for GF was 8 pg on-column. Precision... needs to be addressed with replicate decon solution prep using deactivated RSDL.

Keywords: CWA, GF, Cyclohexyl sarin, decontamination formulation, liquid chromatography-tandem mass spectrometry (LC-MS/MS)

1. Introduction

Scientific authority to write background to the problem, address the question of how the proposed method compares with previously reported methods and show that significant advances are proposed. Reword and add...

2. Materials and methods

2.1. Reagents

GF was prepared on site at DRDC. HPLC-grade organic solvents methanol and acetonitrile were acquired from ?? (city, prov/state, country). Reagent water was produced in the laboratory using a model # reverse osmosis system from manufacturer (city, prov/state, country). Analytical grade formic acid (xx%) was purchased from manufacturer (city, prov/state, country).

2.2. Preparation of standard solutions

A stock solution of GF was prepared using a Gilson model # automated liquid handler (city, prov/state, country). Describe procedure, including weighing of vials to calculate final concentration. Reword and add...

Dilutions ...

2.3. Preparation of decontamination solutions

RSDL was used in decon experiments as a neat solution. The British Decon formulation was prepared according to…

2.4. Instrumentation

All LC-MS/MS analyses were carried out on an Agilent Technologies 1200 liquid chromatograph (Santa Clara, CA, USA) interfaced to an Agilent Technologies 6410B tandem quadrupole mass spectrometer (Santa Clara, CA, USA). Chromatographic separations were achieved using a Zorbax SB-C18 column (2.1 x 50 mm, 1.8 μm particles, Agilent Technologies, Santa Clara, CA, USA). The column was housed in a thermostated compartment maintained at 30 ^{o}C. Table 1 lists the conditions of the binary gradient elution program with water (containing 5 mM ammonium acetate) and methanol as mobile phases. The LC run time was 11 minutes with a 3 minute post time. The injection volume for all LC-MS/MS analyses was 1 μL. A 3 second needle wash was incorporated using a 1:1:1 mixture of DI water, methanol and isopropanol.

The MS source used was electrospray with Agilent Jet Spray, operated in positive ionization mode. The capillary and nozzle voltages were set to 2500 V and 500 V, respectively. The drying gas temperature was 300 ^{o}C, and the drying gas flow rate was 4 L min^{-1}. The nebulizer pressure was 50 psi, and the sheath gas temperature and flow rate were 250 ^{o}C and 10 L min^{-1}, respectively. The fragmentor and collision energy voltages were optimized by flow injection of standard solutions of the target compounds. Multiple reaction monitoring (MRM) was used for the analysis of GF, triethyl phosphate (TEP) and tripropyl phosphate (TPP). The individual MRM transitions for each compound and the corresponding fragmentor voltages and collision energies are provided in Table 2. A dwell time of 90 ms was used for each MRM transition. The precursor for GF is the [M+NH4]+ ammonium adduct while the [M+H]+ precursor is used for TEP and TPP. Time segments were used to divert the LC flow to waste from 0 to 3.5 minutes and 8.5 to

11 minutes. The electron multiplier voltage was increased from the tune value by 200 V for the analytical time segment between 3.5 – 8.5 minutes. High purity nitrogen was used as the collision cell gas. Quantitation was performed using MassHunter Quantitative Analysis software, version B.01.04 (Agilent Technologies, Santa Clara, CA, USA). Concentrations and recoveries were calculated using external standard calibration and a single point calibration.

3. Calculation

3.1. Method development

The analytical method was developed by first optimizing the MS conditions for each compound. TEP and TPP were chosen as surrogate compounds due to their structural similarity to GF. Source parameters were optimized to provide the best drying efficiency for the chromatographic settings used, as measured by the response of GF.

Matrix effects due to the decon solutions were investigated by spiking known amounts of GF, TEP and TPP into increasing dilutions of RSDL and British Decon formulations. The dilutions were analysed and MRM response of each compound was compared to a standard prepared in acetonitrile (ACN) at the same level. Add more...

Chromatography – NH4Ac concentration effects: reduced ion suppression for Br Decon, also reduced ESI signal. Discuss [M+H]+ vs [M+NH4]+ for GF – same ratio throughout experiments, NH4 adduct more sensitive.

Discuss specificity – any observed signal from matrix or added compounds? "The target peaks are well retained and have low background signal with no visible interferences." Check this statement for GF qualifier. Quant and Qual transitions, typical

ratios for each compound, identification criteria (match RT by? And ratio within +/- 20%). Reword and add...

Quant using single point 100% standard prepared same day in solvent. Established linearity initially over a wide range and to determine IDLs to see how low we can go. Reword and add...

3.2. Decon Experiment Considerations

Discuss matching of agent amount to decon solution amount and dilutions required to eliminate ion suppression and still have high enough agent levels to detect down to 1% of original concentration. Reword and add...

4. Results and discussion

4.1 Method performance

Matrix effects – summarize findings from RSDL and Br Decon in terms of amount of dilution required.

TEP elutes prior to GF while TPP elutes after GF. It was found that both TEP and TPP showed different amounts of ion suppression compared to GF. TEP showed more ion suppression than GF in RSDL.... . Discuss TEP and TPP vs GF signal in matrix (TEP good for instrumental analysis to show ion suppression for RSDL, TPP good surrogate to monitor recovery through sample preparation steps.

Discuss NH4Ac concentration effects: reduced ion suppression for Br Decon, also reduced ESI signal. Therefore better to dilute matrix out until no ion suppression detected. Reword and add...

Discuss RT stability, even in matrix. Reword and add...

4.2 Decon Experiment results

Discuss deactivated RSDL and Br Decon mimic to prove that agent would show in matrix. Show table with recoveries for the last experiments showing good TEP and TPP recovery indicating good sample prep and good instrumental method. Reword and add...

5. Conclusions

The analytical method developed was fit for the purpose of analysing GF agent in decontamination formulations. Sufficient dilution of the decon experiment samples was required to eliminate ion suppression. The specificity and sensitivity of the MS/MS allowed detection of the agent in diluted decon experiment samples without any other sample preparation. Reword and add...

Acknowledgements

The authors gratefully acknowledge the assistance of Dr. Paul D'Agostino of DRDC and Ralph Hindle of Vogon Laboratory Services.

References

[1] P.A. D'Agostino (2005). Trend in Chromatography, Vol. 1:23-42.

Figure Captions

Figure 1. .

137 Table 1. LC gradient elution program.

Time (min.)	% A (5 mM NH4Ac in water)	% B (0.1% FA in ACN)	Flow Rate (mL min^{-1})
0	20	80	0.3
1.0	20	80	0.3
8.0	0	100	0.3
9.0	0	100	0.5

138 NH4Ac = ammonium acetate, MeOH = methanol

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140 Table 2. MS/MS multiple reaction monitoring parameters.

Compound	Fragmentor voltage (V)	Quantitation MRM		Confirmation MRM	
		Precursor > product	Collision energy (V)	Precursor > product	Collision energy (V)
GF	60	198.1 > 99.0	4	198.1 > 181.1	0
TEP	70	183.1 > 99.0	15	183.1 > 127	6
TPP	58	225.1 > 99.0	12	225.1 > 141	4

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Annex 6 – Generic protocol for performing decon experiments with a decon formulation not previously studied

Literature Review

- Obtain information on the chemical composition of the decon formulation, including solubility / miscibility with common LC solvents, methods of detection (e.g. LC-UV), etc.

Solubility / Miscibility

- Mix the decon formulation with common LC solvents (DI H20, MeOH, ACN) and note physical characteristics such as miscibility and precipitation.
- Choose an appropriate solvent for dilutions in decon experiments based on miscibility and agent compatability (e.g. water or organic based, if organic, LC-MS compatible).

Identify Elution Pattern

- Using existing chromatography*, inject dilutions of the matrix and analyse by UV if possible or full scan MS. Be sure to cover the entire mass range.
- Inject a solvent blank first to be sure of the peaks that belong to the matrix.
- Inject agents to determine overlap with decon formulation matrix.

Matrix Effects

- Spike agent(s) of interest, TEP, TPP in different dilutions of matrix and also in solvent. Analyse by LC-QQQ.* Compare the response to determine matrix effects.
- Establish a minimum dilution of the decon formulation based on matrix effects and sample preparation criteria. Be aware of possible adduct formation for the agent in decon formula (use fast chromatography and full scan to check).

Adjust Chromatography

- Make adjustments*, if needed, to ensure the matrix elutes before end of run.
- If dilution alone will not adequately remove matrix effects, try altering the gradient and/or changing the column (different phase) to pull target compounds away from eluting matrix and reduce matrix effects.

Test Decon Experiment

- Using the Gilson automated liquid handler, perform a test decon experiment. Use a deactivated or mimic decon solution solution if possible to check for matrix effects on agent without deactivation.
- Add appropriate levels of TPP to decon solution and TEP to final dilution.
- Check the vials to be sure they are thoroughly mixed.
- Analyse by LC-QQQ.

Modify Sampling Procedure

- *Skip this step if the test decon experiment results are acceptable.*
- If the results for the agent, TEP and TPP are not consistent and as expected (compared to solvent standard), go back and confirm the Gilson is working well (by weighing deliveries) and look at each vial for physical solution issues.
- Note that performing a decon experiment by hand may help with troubleshooting.

Perform Decon Experiment

- Perform another decon experiment to confirm the final settings.
- Use a deactivated or mimic decon solution as well as the real decon solution.
- Use surrogate and ISTD type compounds** to verify performance, and include method validation parameters (see below).

Method Validation

- Determine carryover by running a blank after the 100% standard and after the spiked matrix samples.
- Determine precision by performing replicate tests in the decon experiment and by running the final dilutions in replicate on the instrument.

Notes:

* Remove the time segments sending the LC to waste to ensure all compounds of interest are detected.

** Add one of the phosphate compounds (e.g. TPP at a concentration high enough to be detected after dilutions) to the decon solution, and monitor the recovery as a surrogate type compound. Add a different phosphate compound (e.g. TEP) to the final dilution and monitor the recovery. Adding it at the end of the analysis is similar to adding an ISTD, however, it is recommended to use ESTD calculation and monitor absolute recoveries of all target compounds.

Annex 7 – Generic protocol for performing decon experiments with a CWA not previously studied

Literature Review

- Obtain information on chemical formula, solubility and stability in various solvents, storage conditions, exisiting MS analysis parameters, etc.
- Decide which solvent to use for dilutions of agent.

QQQ Optimization

- Using a solution of the CWA between 1-10 ng/µL, inject using FIA or fast chromatography and use Optimizer or manual techniques to determine QQQ optimum fragmentor voltage and collision energy for the compound.
- Build an MRM method from an existing method by changing the MRM details from the previous agent to the new one. Ensure dwell times are set properly.

Establish Chromatography

- Using existing chromatography,*inject dilutions of the agent dossolved in a suitable solvent. Confirm that the compound elutes in a suitable chromatographic region. Adjust the gradient if necessary.
- Compare different organic mobile phases (MeOH and ACN).
- Evaluate TEP, TPP and TBP (if needed for a very late eluter) as surrogate or internal standard type compounds.

Determine Linearity, IDL

- Inject 5x dilutions (low to high, approx. 0.0001 - 2 ng/µL) of the agent to determine linearity and the instrument detection limit.
- Establish the approximate range of the compound in final solution to be analysed based on instrument sensitivity** and compatibility with decon experiment protocol (handling of CWA solutions).

Matrix Effects

- Spike agent, TEP and TPP at same level in different dilutions of a deactivated decon formulation of interest (or a mimic solution) and also in solvent (std).
- Run these spikes from low to high concentration of matrix and compare the response to that of the std to determine matrix effects.
- If necessary, check for adduct formation of agent in decon mix (fast chromatography, full scan), especially if decon contains cations like Na, K.

Adjust Chromatography

- Make adjustments*, if needed, reduce matrix effects. Try altering the gradient and/or changing the column (different phase) to pull target compounds away from eluting matrix and reduce matrix effects.

Test Decon Experiment

- Using the Gilson automated liquid handler, perform a test decon experiment. Use a deactivated or mimic decon solution solution if possible to check for matrix effects on agent without deactivation.
- Add appropriate levels of surrogate and ISTD type compounds to decon solution and final dilution, respectively (e.g. TPP and TEP).
- Analyse by LC-QQQ.

Modify Sampling Procedure

- *Skip this step if the test decon experiment results are acceptable.*
- If the results for TEP and TPP are not consistent and as expected (compared to solvent standard), go back and confirm the Gilson is working well (by weighing deliveries) and double checking starting solution concentrations.
- Note that performing a decon experiment by hand may help with troubleshooting.

Perform Decon Experiment

- Perform another decon experiment to confirm the final settings.
- Use a deactivated or mimic decon solution as well as the real decon solution.
- Use surrogate and ISTD type compounds*** to verify performance, and include method validation parameters (see below).

Method Validation

- Determine carryover by running a blank after the 100% standard and after the spiked matrix samples.
- Determine precision by performing replicate tests in the decon experiment and by running the final dilutions in replicate on the instrument.

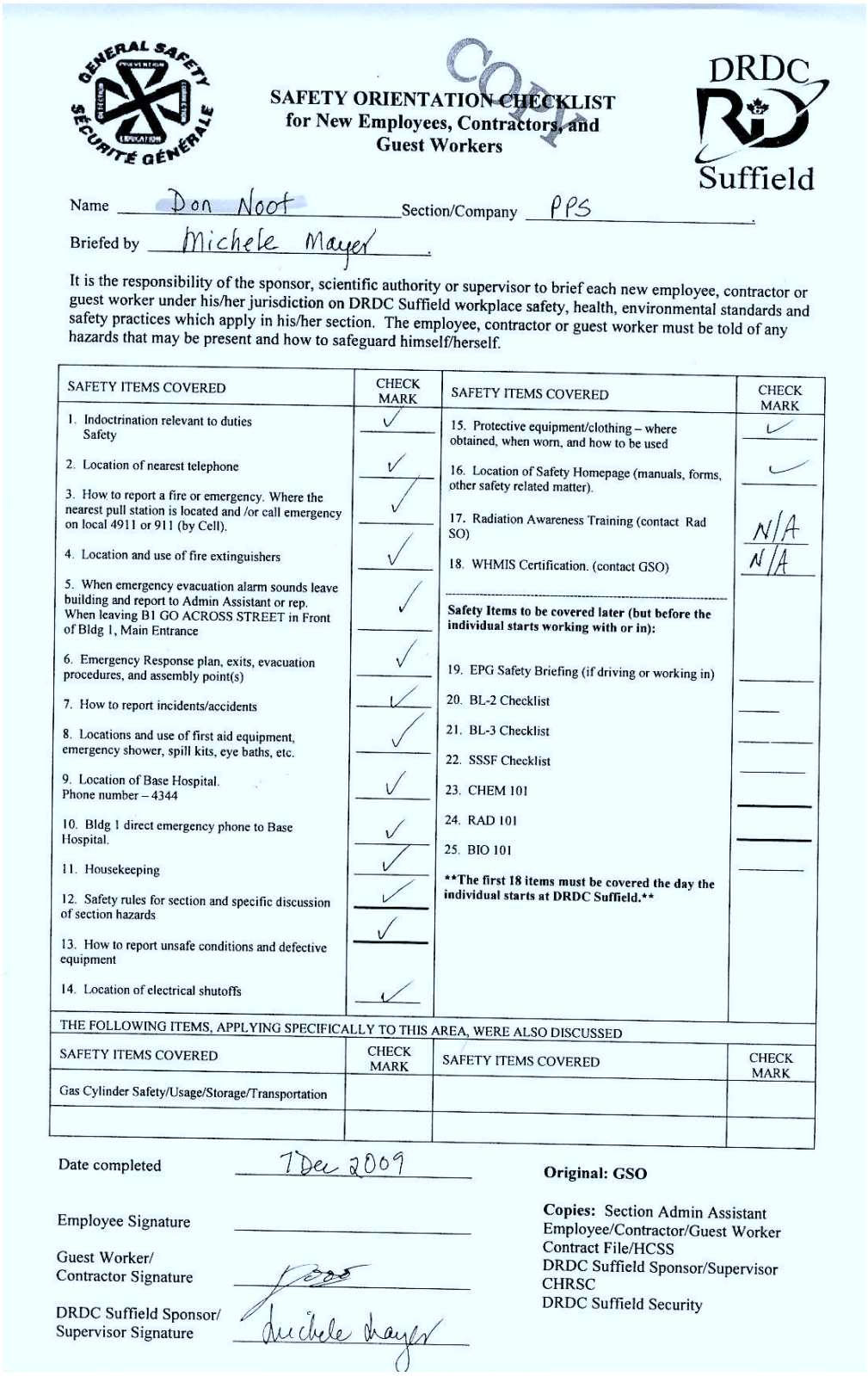
Notes:

* Remove the time segments sending the LC to waste to ensure all compounds of interest are detected.

** The signal of the agent should be high enough to allow detection down to 1% of original concentration with at least 10:1 Signal to Noise.

*** Add one of the phosphate compounds (e.g. TPP at a concentration high enough to be detected after dilutions) to the decon solution, and monitor the recovery as a surrogate type compound. Add a different phosphate compound (e.g. TEP) to the final dilution and monitor the recovery. Adding it at the end of the analysis is similar to adding an ISTD, however, it is recommended to use ESTD calculation and monitor absolute recoveries of all target compounds.

Annex 8 Completed Safety Checklist



SAFETY ORIENTATION CHECKLIST
for New Employees, Contractors, and Guest Workers

COPY

DRDC Suffield

Name Don Noot Section/Company PPS

Briefed by Michele Mayer

It is the responsibility of the sponsor, scientific authority or supervisor to brief each new employee, contractor or guest worker under his/her jurisdiction on DRDC Suffield workplace safety, health, environmental standards and safety practices which apply in his/her section. The employee, contractor or guest worker must be told of any hazards that may be present and how to safeguard himself/herself.

SAFETY ITEMS COVERED	CHECK MARK	SAFETY ITEMS COVERED	CHECK MARK
1. Indoctrination relevant to duties Safety	✓	15. Protective equipment/clothing – where obtained, when worn, and how to be used	✓
2. Location of nearest telephone	✓	16. Location of Safety Homepage (manuals, forms, other safety related matter).	✓
3. How to report a fire or emergency. Where the nearest pull station is located and /or call emergency on local 4911 or 911 (by Cell).	✓	17. Radiation Awareness Training (contact Rad SO)	N/A
4. Location and use of fire extinguishers	✓	18. WHMIS Certification. (contact GSO)	N/A
5. When emergency evacuation alarm sounds leave building and report to Admin Assistant or rep. When leaving B1 GO ACROSS STREET in Front of Bldg 1, Main Entrance	✓	**Safety Items to be covered later (but before the individual starts working with or in):**	
6. Emergency Response plan, exits, evacuation procedures, and assembly point(s)	✓	19. EPG Safety Briefing (if driving or working in)	
7. How to report incidents/accidents	✓	20. BL-2 Checklist	
8. Locations and use of first aid equipment, emergency shower, spill kits, eye baths, etc.	✓	21. BL-3 Checklist	
9. Location of Base Hospital. Phone number – 4344	✓	22. SSSF Checklist	
10. Bldg 1 direct emergency phone to Base Hospital.	✓	23. CHEM 101	
11. Housekeeping	✓	24. RAD 101	
12. Safety rules for section and specific discussion of section hazards	✓	25. BIO 101	
13. How to report unsafe conditions and defective equipment	✓	****The first 18 items must be covered the day the individual starts at DRDC Suffield.****	
14. Location of electrical shutoffs	✓		

THE FOLLOWING ITEMS, APPLYING SPECIFICALLY TO THIS AREA, WERE ALSO DISCUSSED

SAFETY ITEMS COVERED	CHECK MARK	SAFETY ITEMS COVERED	CHECK MARK
Gas Cylinder Safety/Usage/Storage/Transportation			

Date completed 7 Dec 2009

Employee Signature

Guest Worker/ Contractor Signature

DRDC Suffield Sponsor/ Supervisor Signature Michele Mayer

Original: GSO

Copies: Section Admin Assistant
Employee/Contractor/Guest Worker
Contract File/HCSS
DRDC Suffield Sponsor/Supervisor
CHRSC
DRDC Suffield Security

Annex 9 – Monthly Reports for Dec 2009, Jan & Feb 2010

VLS Vogon Laboratory Services Ltd.
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Monthly Status Report – Development of LC/MS Methods

contract # W7702-09R230

For the time period ending Dec. 24, 2009

Hours spent on the project

Since previous report – 9.4 days

Total accumulated for the project – 9.4 days

Activities during the reporting period

General

- Building and safety orientation
- Investigate instrument issues: LC pressure fluctuation and MS noise

Objective 1

- Literature review
- Optimize 6410 QQQ parameters for GF using ESI
- Initial chromatography conditions for GF and internal standard TEP
- Initial optimization for GD
- Initial assessment and optimization of APCI for GF
- Initial investigation of mPEG "solvent" in decon solution using DAD

Objective 2

- none

Summary of accomplishments

- ESI and chromatographic conditions established for GF. Using ammonium acetate produces a strong ammonium adduct that provides increased sensitivity and consistent spectra and MRM transitions.
- Instrument Detection Limits and linearity established for GF.
- Initial instrumental parameters obtained for GD.
- Initial parameters for APCI obtained.
- Instrument issues resolved: backflushed LC autosampler needle seat to stabilize pressure and MS power-cycled to reduce noise to normal levels.

Issues that would impact completion of the project

- Optimizing the instrument conditions for the CW agents has proved to be rather complicated and will take more time than initially expected. Project targets should still be able to be met, however, within the project timelines.

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Monthly Status Report – Development of LC/MS Methods
contract # W7702-09R230
For the time period - January, 2010

Time spent on the project

Since previous report: 10.5 days
Total accumulated for the project: 20.2 days
Estimated Remaining for the project: 16.8 days

Executive Summary

General

- Safety briefing on medical countermeasures when using CW agents.
- Instrument issue: LC pressure fluctuation remedied by installing new autosampler needle seat

Objective 1 – actions and results

- Optimize chromatography conditions for GF and internal standard TEP – best mobile phase: A=5 mM ammonium acetate; B = MeOH.
- Comparison of ESI and APCI for TEP and GF – although both show similar detectability, ESI provides better precision.
- Characterization of F54 matrix and evaluation of matrix effects on GF and TEP using ESI and APCI – LC stop time adjusted to 10 minutes to ensure all F54 components elute; matrix effects (ion suppression) occur at specific dilutions in ESI, fewer matrix effects in APCI (preliminary); extended chromatography reduces matrix effects.
- Investigation of mPEG matrix effects on GF and TEP using ESI – significant ion suppression observed in ESI.

Objective 2– actions and results

- Review past decon experiment conditions and modify them based on optimized 6460 method and observed matrix effects – spreadsheet developed to provide ratios of decon to agent and volumes to use for diluted and neat decon solutions.
- Test new decon experiment design using F54 and GF as a model – five variations of British Decon tested and dilutions analysed, some modifications needed based on variability in the results.

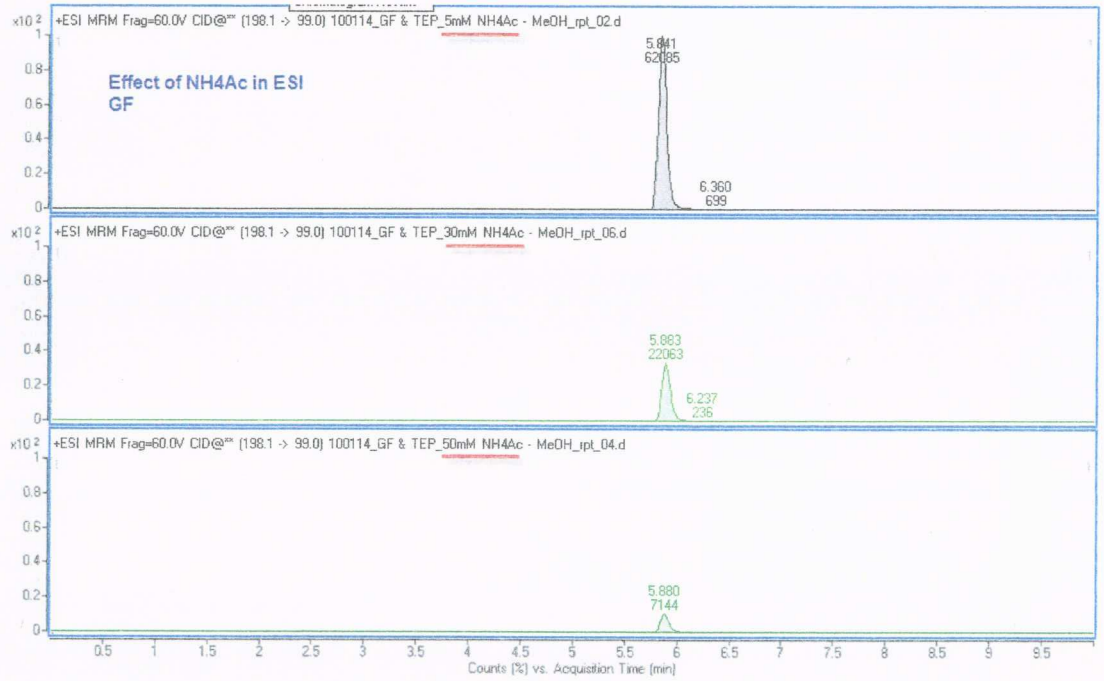
Issues that may impact completion of the project

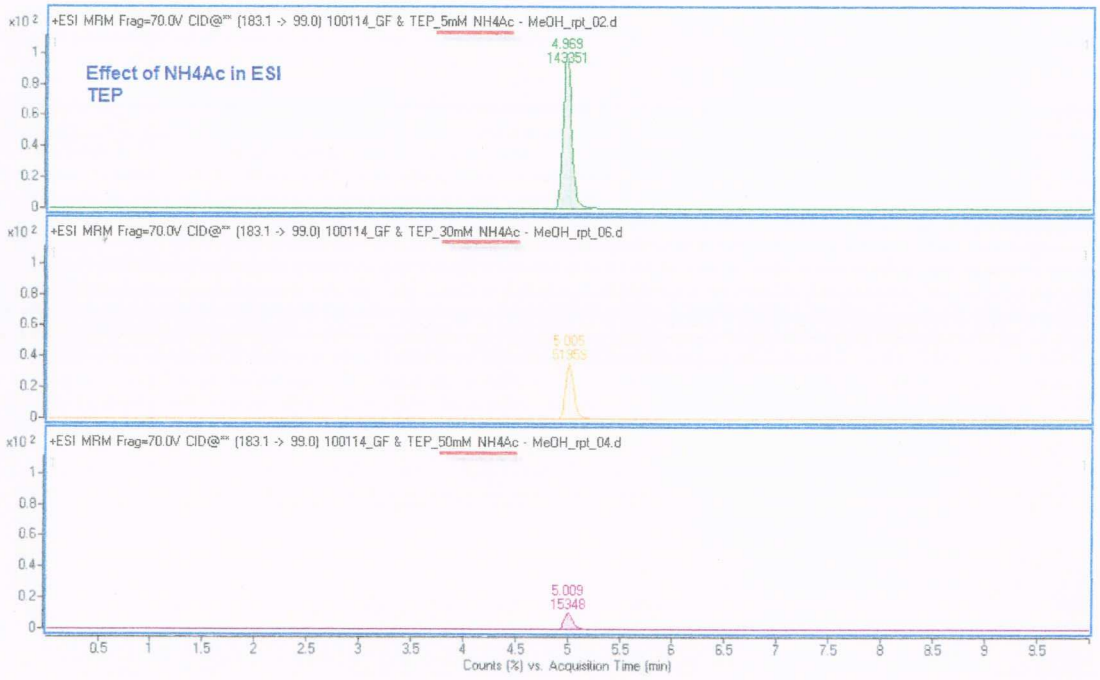
- Work performed on determining matrix effects of two different decon solutions (F54 and MPEG) has shown that sufficient dilution is critical to reducing ion suppression from the matrix when analyzing solutions directly by LC-MS. This is an ion source phenomenon, and as such, would apply equally to the 6130 single quad mass spectrometer as well as the 6460 triple quad system. The 6130 MS is less sensitive and less specific than the 6460, and as such, it would not likely provide useful analytical results. Use of the 6130 instrument would require some form of sample manipulation other than dilution prior to analysis, and this would fall outside the scope of this project. Therefore, specific tasks for both Objectives 1 & 2 related to developing a method on the 6130 may not be performed.

Detailed Accomplishments and Results

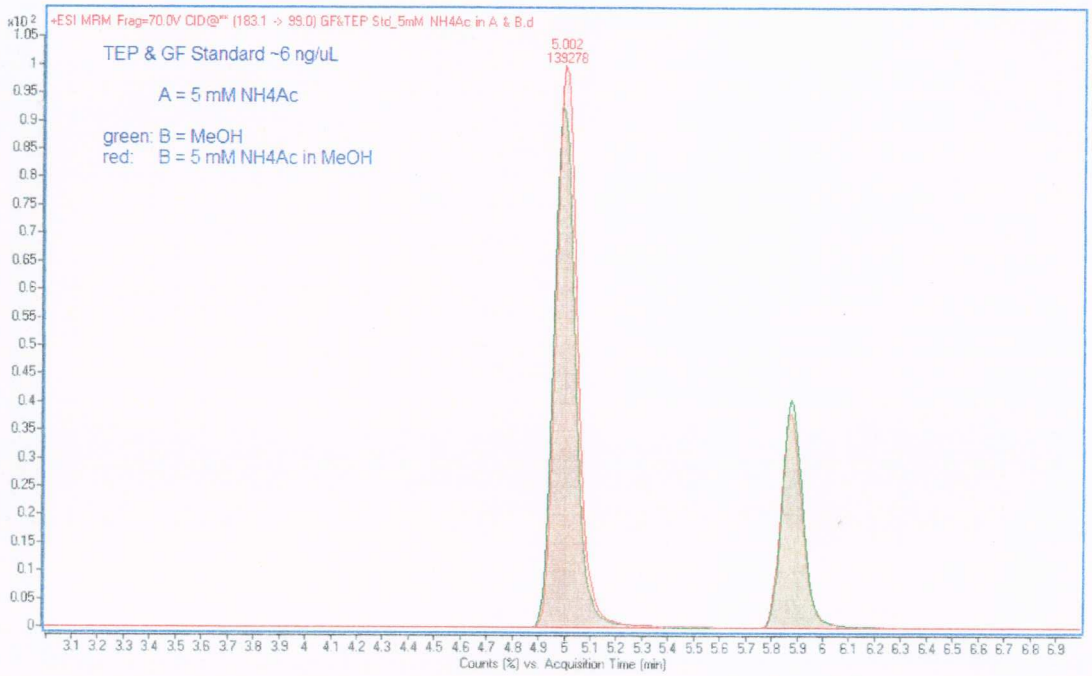
Objective 1

1. Optimize chromatography conditions for GF and internal standard TEP.
    - The response of GF and TEP showed significant reduction with increasing ammonium acetate (NH_4Ac) concentration.

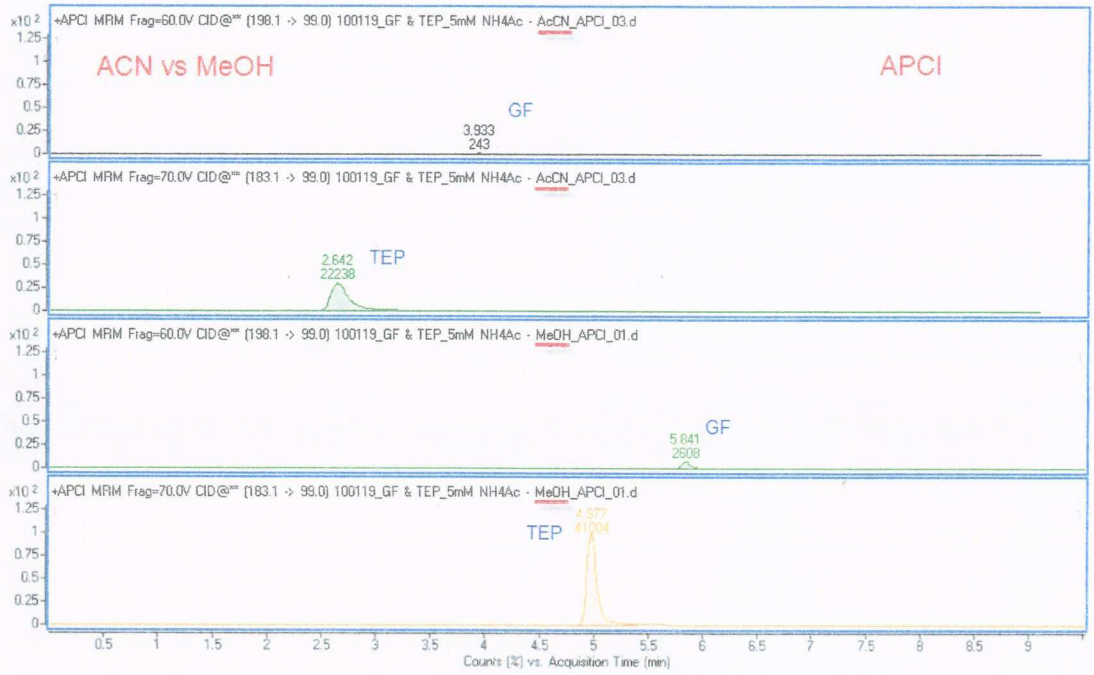


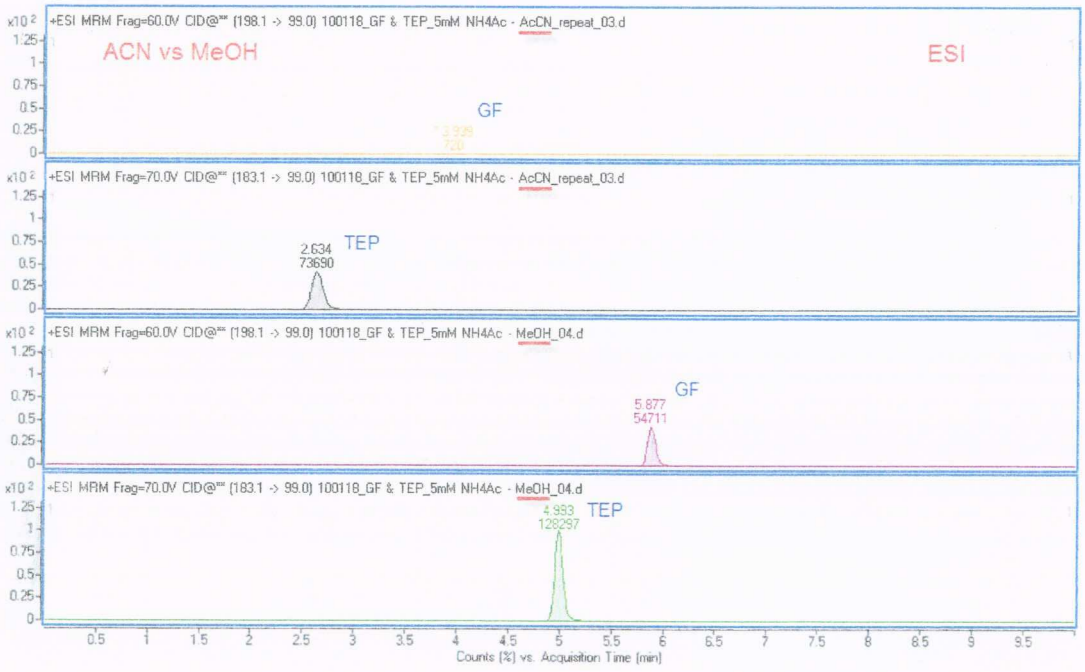


- Adding NH_4Ac to the B mobile phase (methanol) does not provide significant improvement, therefore add NH_4Ac to A mobile phase only.

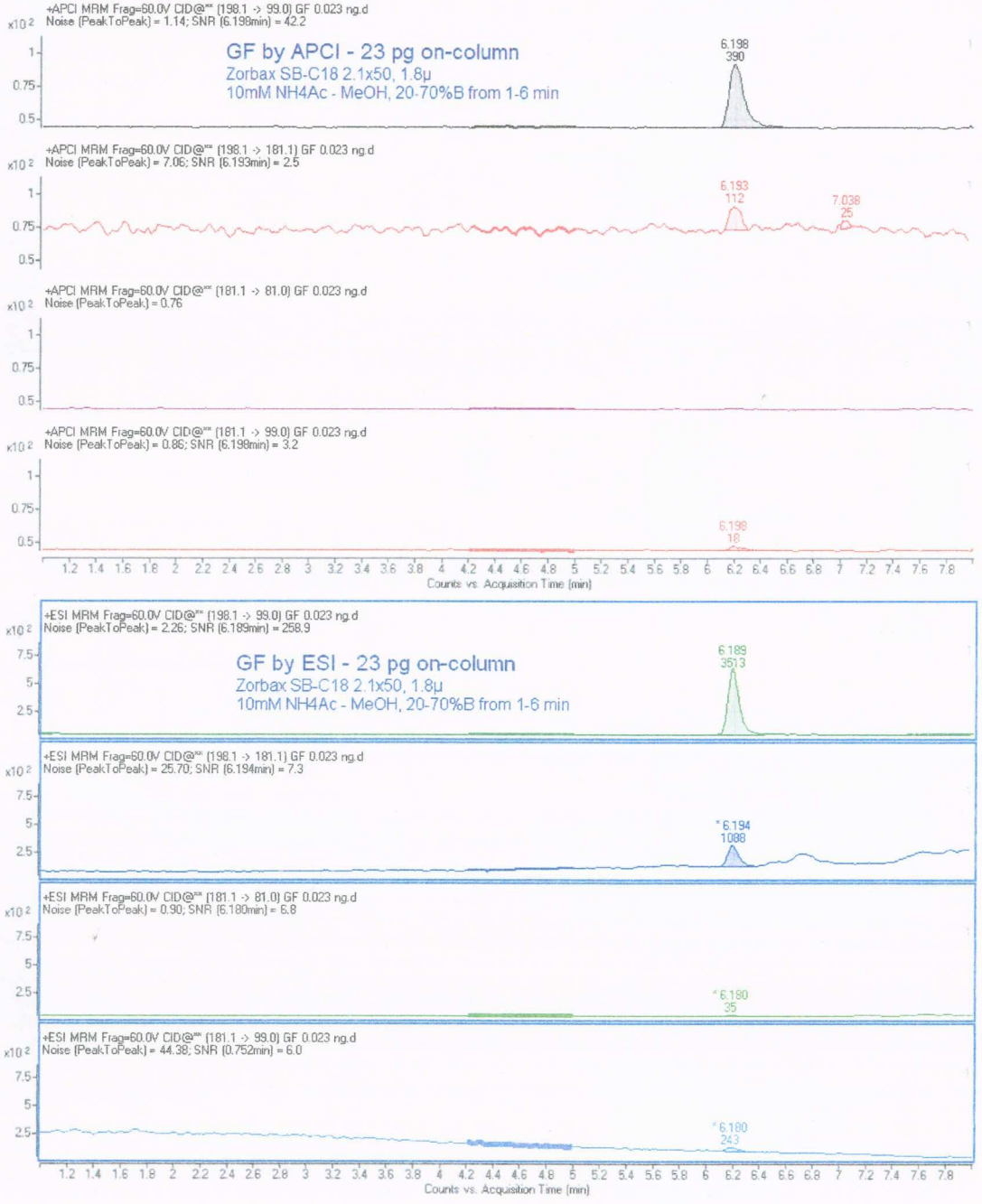


- Methanol and acetonitrile were compared as mobile B in both APCI and ESI. TEP response was reduced in ACN by almost 50% whereas GF was reduced by more than 90%, therefore MeOH is optimal.



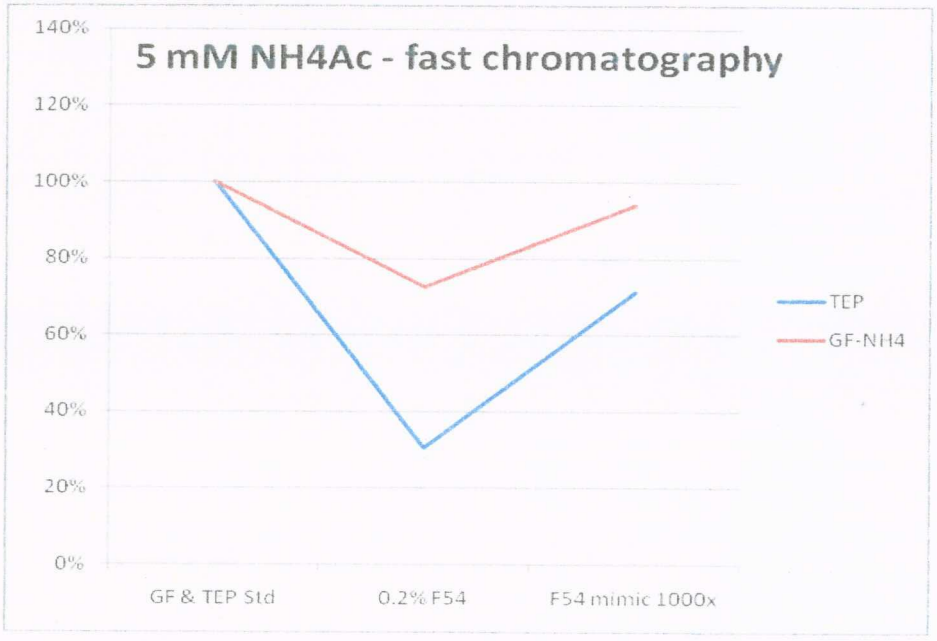


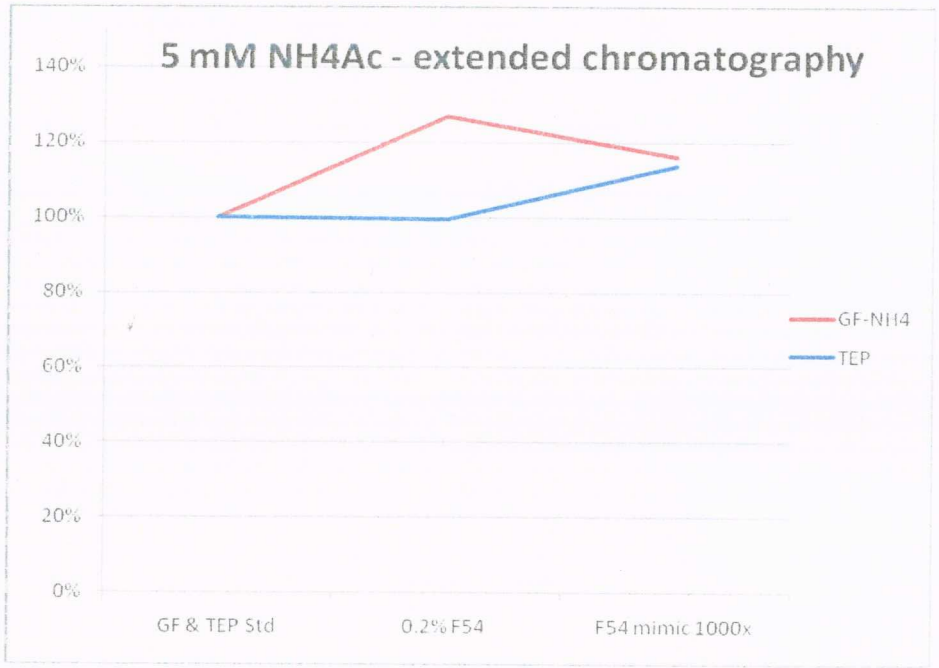
2. Comparison of ESI and APCI for TEP and GF.
    - Instrument detection limits were compared for GF & TEP using APCI and ESI. ESI with Agilent Jet Stream showed higher area counts yet detectability for both quantifier and qualifier transition is similar for both techniques at approximately 0.023 ng (23 pg) on-column.



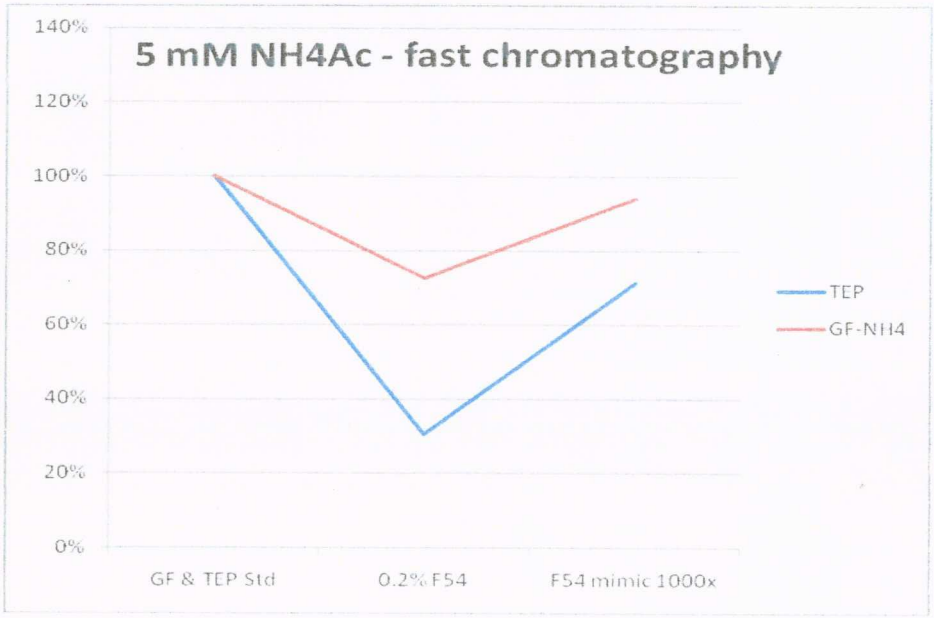
    - Preliminary results investigating matrix effects with F54 and GF & TEP using APCI showed that this source may be less susceptible to ion suppression than ESI. With appropriate chromatography and F54 dilutions, ESI, however, has been shown to work very well and give better precision for GF & TEP. The use of ESI is therefore recommended for F54 based solutions and these targets.

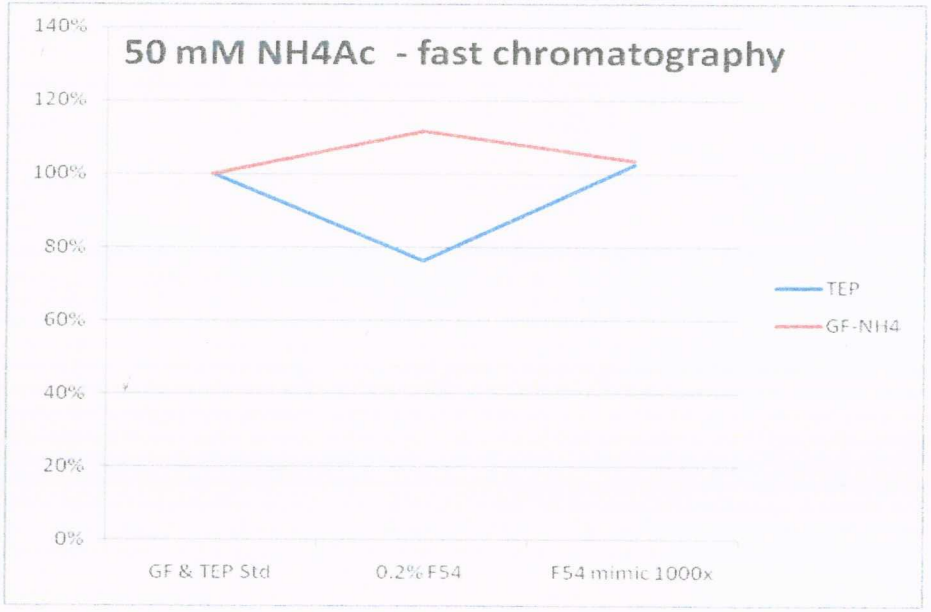
3. Characterization of F54 matrix and evaluation of matrix effects on GF and TEP using ESI and APCI.
    - F54 matrix caused ion suppression when using fast chromatography (little separation) in ESI, however the developed (extended) chromatographic method reduced the suppression, even with relatively high levels of matrix present.



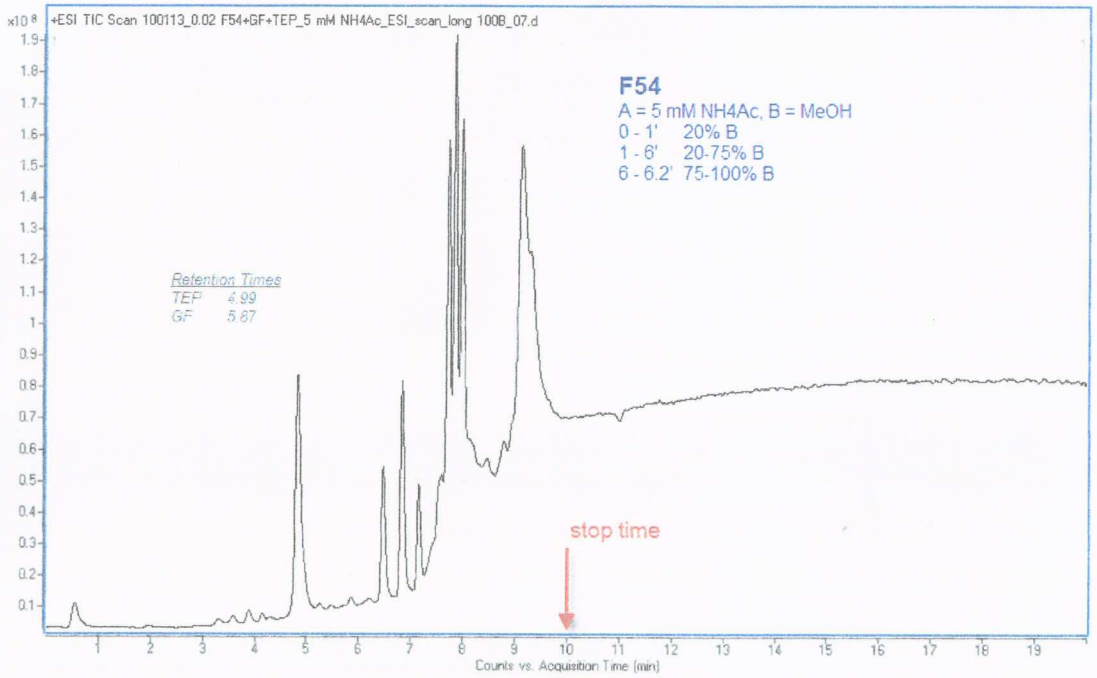


- Increasing concentrations of NH_4Ac reduced matrix effects of F54 on GF and TEP in ESI. However, as stated in point #1 above, ESI signal is severely reduced as NH_4Ac concentration increases. Therefore, use of 5mM NH_4Ac for ESI is recommended.

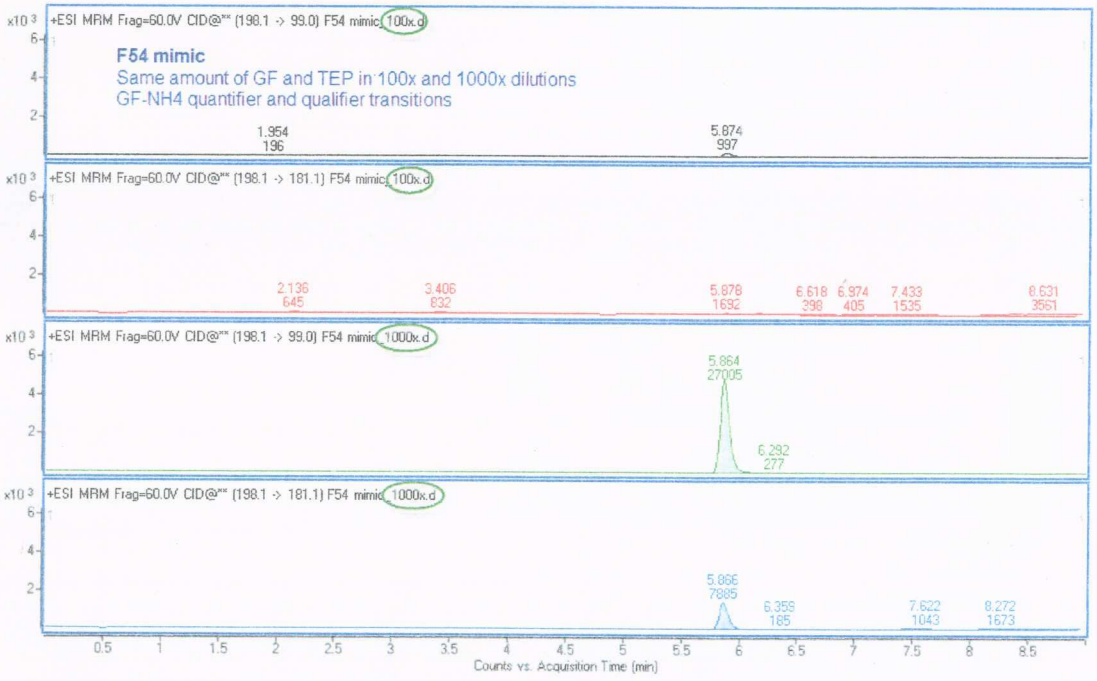


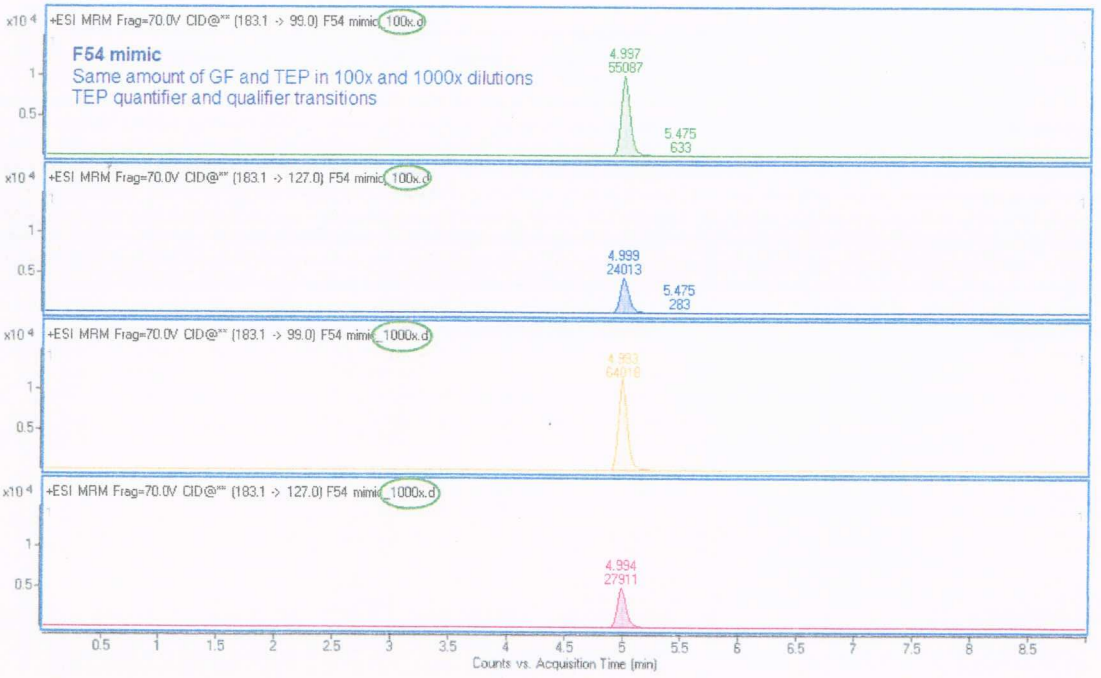


- F54 matrix was analysed in full scan and the LC stop time adjusted to allow for all components to elute. 10 minutes is required using existing gradient.

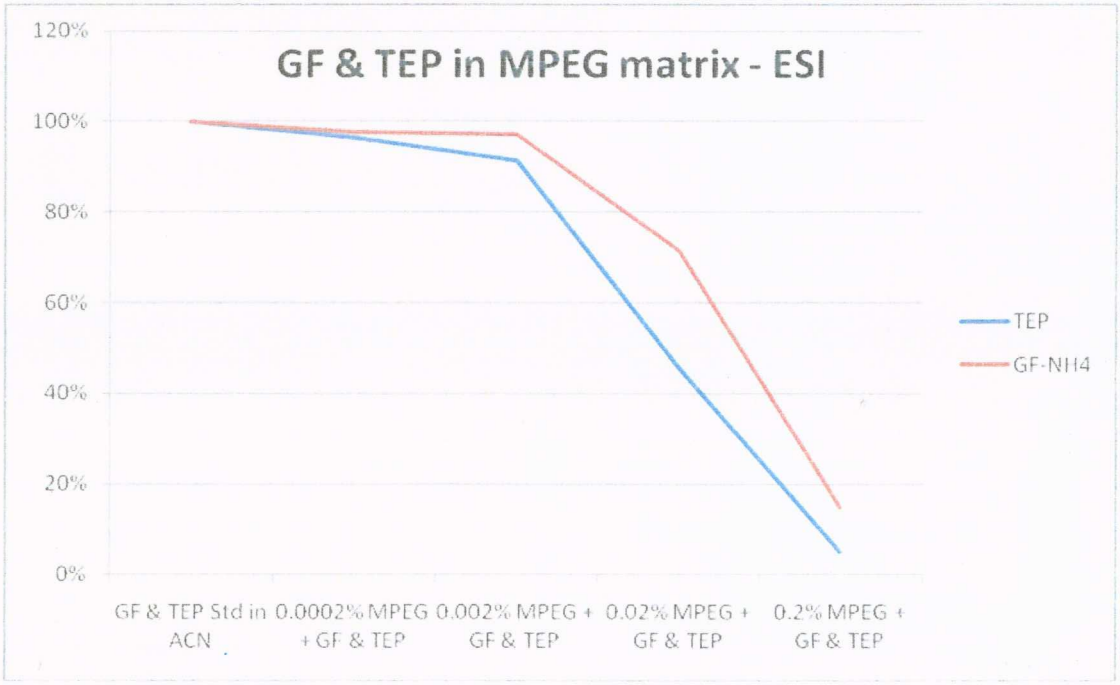


- A solution mimicking the British Decon solution was made by using sodium carbonate in place of sodium percarbonate, as this provides the sodium content without the oxidizing potential. Analysis of various dilutions of F54 with Na_2CO_3 showed that at the lowest level of dilution tested (most concentrated F54), the signal of GF was significantly reduced while TEP was not significantly affected. This data was used in the decon experiment design to ensure the matrix is sufficiently diluted (to at least 1000x, corresponding to 0.02% F54) to avoid matrix effects.





4. Initial investigation of mPEG matrix effects on GF and TEP using ESI.
    - Analysis of GF & TEP in diluted MPEG 550 by ESI showed significant ion suppression at low levels of dilution. More work will need to be done to either reduce the matrix effects or ensure that experiments with RSDL are performed at an appropriate dilution where the effects are acceptable.



Objective 2

5. Review past decon experiment conditions and modify them based on optimized 6460 method and observed matrix effects.
    - The previous Decon experiment design was reviewed and modified based on the information gathered to date. A spreadsheet was developed for the preparation of diluted and neat decon solutions, the amount of agent to use and appropriate dilutions prior to analysis on the 6460 LC-MS/MS instrument.

6. Test the new decon experiment design using F54 and GF as a model.
    - The Decon experiment spreadsheet was tested using British Decon and F54 with GF and TEP as an internal standard. Results of this initial test showed some unexpected variability for TEP as well as GF. Initial attempts at determining the cause of this variability (matrix interference, solution preparation, etc.) were made. Further work is necessary to determine the exact cause and, if necessary, modify the analytical testing or experimental design.

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Monthly Status Report – Development of LC/MS Methods
contract # W7702-09R230
For the time period - February, 2010

Time spent on the project

Since previous report: 4.8 days
Total accumulated for the project: 25 days
Estimated Remaining for the project: 12.0 days

Executive Summary

General

- Discussed options for obtaining security clearance which may be necessary for ongoing work.

Objective 1 – actions and results

- Review and optimize the sampling procedure - this involved tracking down non-reproducible results in the decon experiments performed to date. This was necessary to determine if the cause of the problem was related to the LC-MS/MS method that was developed during this project or the sample preparation procedure. It was determined that there are physical issues with mixing the decon solutions and that use of the Gilson automated liquid handling device alone in decon experiments is not effective.
- Investigate chromatography – columns of different dimensions were investigated for use in analysis by APCI, and reproducibility of replicate standard injections was performed.
- Optimize APCI-LC-QQQ conditions – conditions for GF were optimized at the higher flow rates of larger dimension columns.
- Determine ion suppression from RSDL - reviewed data files from MPEG dilutions analysed by APCI. APCI shows fewer matrix effects than ESI.

Objective 2– actions and results

- Document step by step process (work flow) for starting work with a new decon material and new agents – investigated options for presenting this material using Microsoft Word.

Issues that may impact completion of the project

- In discussions with the scientific authority (Michele Mayer), it was decided that the single quad (6130) instrument will not be suitable for use in this project, and therefore all actions related to this item were dropped. See the updated Gant chart for details.

- Much of the time in February was spent reviewing and optimizing the sampling procedure (objective 1 – see above). This troubleshooting exercise took significant time which was not scheduled. As such, it may impact completion of other activities in the project.

Detailed Accomplishments and Results

Objective 1

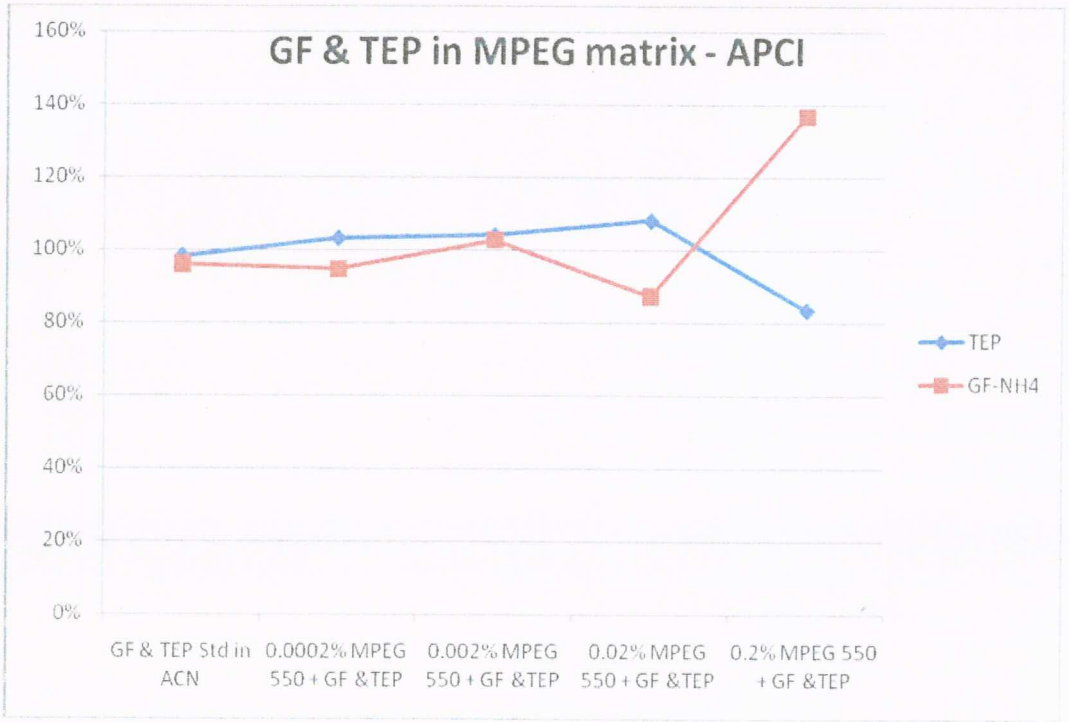
1. Review and optimize the sampling procedure. Several experiments were performed on the Gilson automated liquid handling instrument using decon solutions. Some solutions (e.g. containing F-54 in water) form a micro-emulsion and then separate over time. Others are very viscous (e.g. containing MPEG found in RSDL) and the Gison default parameters for mixing steps are ineffective. Different mixing procedures and settings on the Gilson system were investigated and compared to manual mixing and totally manual solution preparation procedures. Results of this testing indicate that, when using the Gilson system, a manual-mixing-by-hand step should be incorporated after the decon solutions are prepared and before they are sub-sampled. Procedures were built on the Gilson system that incorporate wait steps that allow hand-mixing.

2. Investigate chromatography. Two different columns were investigated: Cogent Bidentate C18, 2.1x75mm 4u and a Zorbax Eclipse XDB-C18, 4.6x50mm, 1.8u. Both of these columns have less back pressure than the Zorbax SB-C18 column (2.1x50mm, 1.8u) used to date which allowed investigation of higher flow rates with APCI. All three columns provided similar separation of TEP and GF.

3. Optimize APCI-LC-QQQ conditions. Source conditions for use with higher column flow rates were optimized. Reproducibility of APCI was then checked at these higher flow rates. Reproducibility was similar at flow rates of 0.3 and 1 mL/min.

0.3 mL/min flow rate			**1 mL/min flow rate**		
GF-NH4	GF	TEP	GF-NH4	GF	TEP
13.7%	12.8%	3.3%	12.6%	10.3%	5.0%

4. Determine ion suppression from RSDL. TEP and GF in dilutions of MPEG solution were run and the data reviewed. APCI was shown to be less susceptible to matrix effects as compared to ESI. Also, where the increasing concentration of MPEG caused ion suppression is ESI, some degree of ion enhancement was noted for APCI.



Objective 2

5. Document step by step process (work flow) for starting work with a new decon material and new agents. Various options were investigated for presenting work flow diagrams in Microsoft Word.

DOCUMENT CONTROL DATA

(Security classification of title, body of abstract and indexing annotation must be entered when the overall document is classified)

1. ORIGINATOR (the name and address of the organization preparing the document. Organizations for who the document was prepared, e.g. Establishment sponsoring a contractor's report, or tasking agency, are entered in Section 8.)

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   Unit 104, 90 Freeport Blvd NE
   Calgary, AB T3J 5J9

2. SECURITY CLASSIFICATION
   (overall security classification of the document, including special warning terms if applicable)

   Unclassified

3. TITLE (the complete document title as indicated on the title page. Its classification should be indicated by the appropriate abbreviation (S, C or U) in parentheses after the title).

   Development of LC/MS Methods to be used in Decontamination Research with CW Agents

4. AUTHORS (Last name, first name, middle initial. If military, show rank, e.g. Doe, Maj. John E.)

   Noot, Don

5. DATE OF PUBLICATION (month and year of publication of document)

   June 2010

6a. NO. OF PAGES (total containing information, include Annexes, Appendices, etc) 194

6b. NO. OF REFS (total cited in document) 4

7. DESCRIPTIVE NOTES (the category of the document, e.g. technical report, technical note or memorandum. If appropriate, enter the type of report, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.)

   Final Contract Report (Dec 2009 to March 2010)

8. SPONSORING ACTIVITY (the name of the department project office or laboratory sponsoring the research and development. Include the address.)

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9a. PROJECT OR GRANT NO. (If appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant.)

9b. CONTRACT NO. (If appropriate, the applicable number under which the document was written.)

   W7702-09R230

10a. ORIGINATOR'S DOCUMENT NUMBER (the official document number by which the document is identified by the originating activity. This number must be unique to this document.)

   DRDC Suffield CR 2010-147

10b. OTHER DOCUMENT NOs. (Any other numbers which may be assigned this document either by the originator or by the sponsor.)

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Analytical methods using liquid chromatography-tandem mass spectrometry for the detection of CWAs in decontamination formulations were developed and validated. Various parameters were investigated, including mass spectrometer parameter optimization, investigation of ionization matrix effects, chromatographic separation, use of internal standard type compounds, linearity, carry over and precision. The sampling design for decon experiments was also investigated and modified to ensure accurate results. The methods are suitable for the agents GF and GD, and the decon formulations RSDL and British Decon using F54.

The final methods allow detection of agents in decon formulation samples using dilution as the only sample preparation step ("dilute and shoot"). As such, the methods will provide accurate identification and quantitation of agents in real time to test decon formulation efficacy.

Generic protocols for adapting the developed methods for use with other agents and decon formulations were also prepared.

14. KEYWORDS, DESCRIPTORS or IDENTIFIERS (technically meaningful terms or short phrases that characterize a document and could be helpful in cataloguing the document. They should be selected so that no security classification is required. Identifies, such as equipment model designation, trade name, military project code name, geographic location may also be included. If possible keywords should be selected from a published thesaurus, e.g. Thesaurus of Engineering and Scientific Terms (TEST) and that thesaurus-identified. If it is not possible to select indexing terms which are Unclassified, the classification of each should be indicated as with the title.)

Method development
Liquid chromatography
Tandem mass spectrometry
GF
GD
Decontamination
RSDL
Chemical warfare agent

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Science and Technology

R & D pour la défense Canada

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